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ABSTRACT

This book is one in a series of Interdisciplinary Approaches to Chemistry (IAC). The purpose of this guide is to familiarize students with chemistry and its everyday applications around the world using inquiry and investigations. Contents include: (1) "Considering Life Processes"; (2) "Understanding the Structure of Biomolecules"; (3) "Properties and Reactions of Biomolecules"; (4) "Enzymes: Where the Action Is?"; (5) "Metabolism: The Community of Enzyme Reactions"; (6) "The Organization of Cellular Activities"; and (7) "Where Are We?" (YDS)

interdisciplinary approaches to chemistry

IAC

MOLECULES IN LIVING SYSTEMS

A BIOCHEMISTRY MODULE

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MOLECULES IN LIVING SYSTEMS

A BIOCHEMISTRY MODULE

David Martin
Joseph Sampugna

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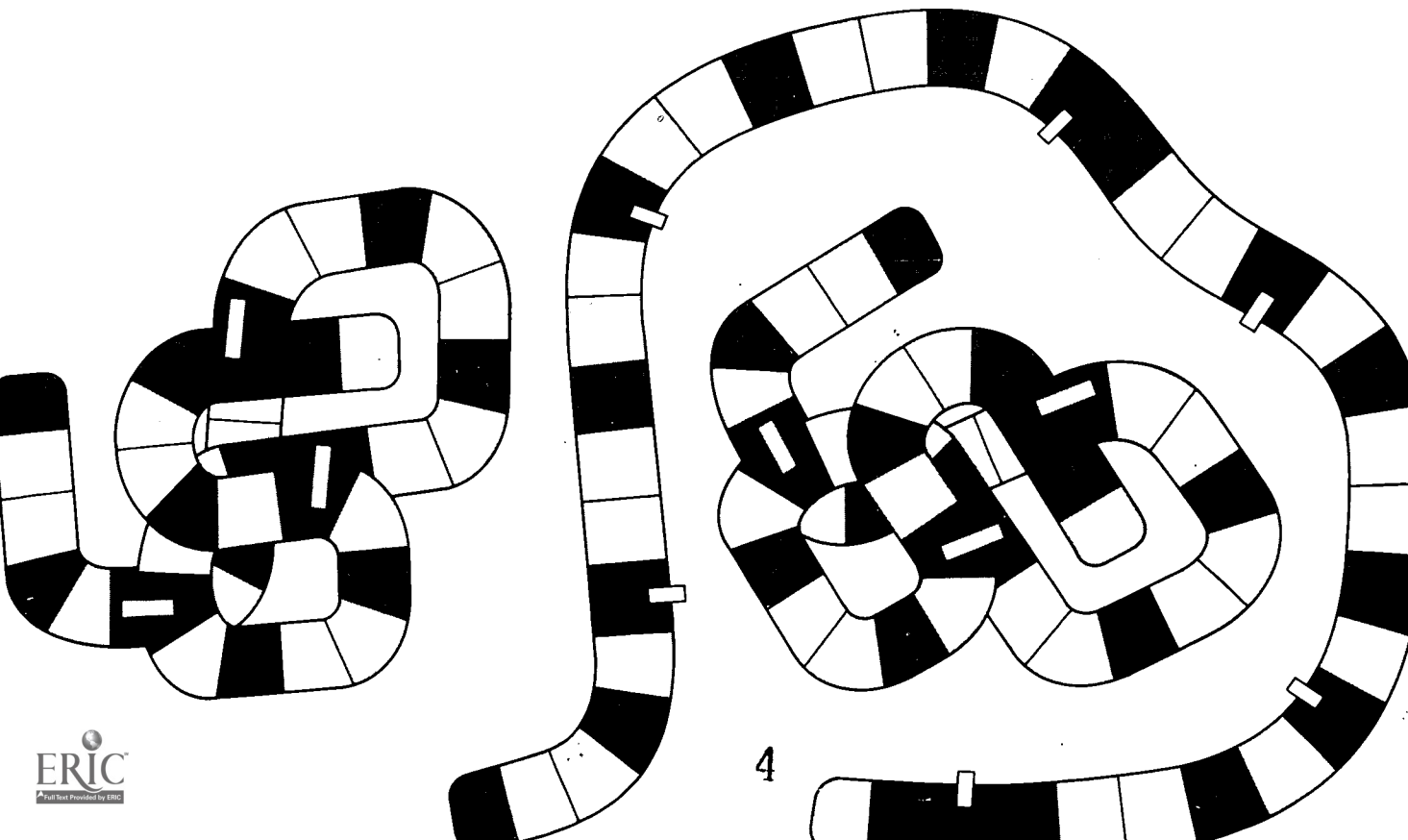

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Directors of IAC:
Marjorie Gardner, 1971–73, 1976–
Henry Heikkinen, 1973–76

Revision Coordinator:
Alan DeGennaro

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AUTHORS

MOLECULES IN LIVING SYSTEMS:
A BIOCHEMISTRY MODULE

David Martin and Joseph Sampugna

DAVID MARTIN

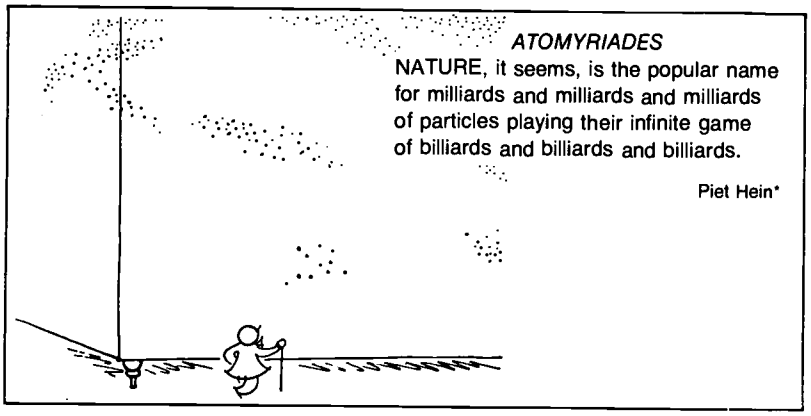
David Martin received his Ph.D. in biochemistry from the University of Wisconsin. Since his arrival at the University of Maryland in 1968, he has been actively engaged in pursuing his research interests and deeply involved in the teaching program of the Chemistry Department. His research deals with the biochemical processes involved in the transmission of nerve impulses between nerve cells in the central nervous system.

In addition to a hectic research schedule and undergraduate and graduate teaching duties, David has been actively involved with the IAC program both as author and policy maker since its inception. Although these obligations would be enough to keep most people busy, he still finds time to pursue his favorite pastime of photography and to go cross-country skiing and camping with his family.

JOSEPH SAMPUGNA

Joseph Sampugna completed his graduate training at the University of Connecticut, where he received an M.A. in psychology in addition to a Ph.D. in biochemistry. He joined the Chemistry Department faculty at the University of Maryland in 1968. Joe has taught at both the graduate and undergraduate levels, including courses in general chemistry, biochemistry, lipids, and neurochemistry. His research interests are in lipid biochemistry and involve studies of membranes isolated from brain tissue.

Joe is an avid bowler and sports fan. His voice can be heard cheering the University of Maryland Terrapin football and basketball teams at all of their home games.



PREFACE

Welcome to IAC Chemistry. Enjoy this year as you explore this important area of science. Chemistry is to be enjoyed, cultivated, comprehended. It is part of our culture, of our everyday lives.

Polymers, paints, pharmaceuticals, people, and pollution all have something in common—a chemical base. IAC Chemistry is relevant, interdisciplinary, student centered, and filled with important concepts and processes.

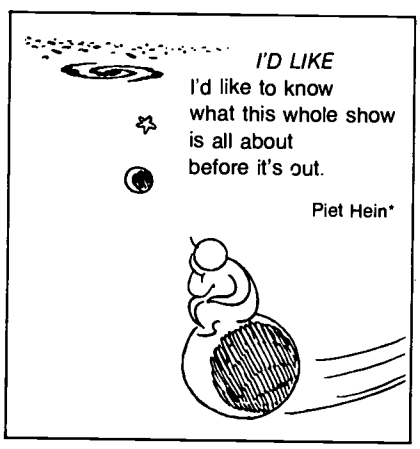
IAC will help you discover that chemistry is a lively science and being actively used to pursue solutions to the important problems of today. You will see how chemistry is taking place continuously all around. You will more readily understand the daily problems facing you and your environment.

Students throughout this country and in a number of other countries as well have let us know that they like and learn from the IAC modules. Classroom teachers have suggested changes to make them even better.

Since the IAC authors believe that student involvement in chemistry is very important, there are many activities that allow you to develop and apply chemistry concepts directly. We have tried to make the modules flexible, easy to read, and enjoyable, discussing everyday problems and adding a bit of humor that may help you remember some of the more important ideas. The Time Machines are intended to give you a sense of when the more important discoveries in chemistry happened in relation to other events.

Wonder—inquire—investigate. Think through all that you find here. But most of all—enjoy chemistry as you learn about the atoms, molecules, elements, and compounds that make up your universe. IAC is written for your learning pleasure.

Marjorie Gardner
Director
Interdisciplinary Approaches to Chemistry



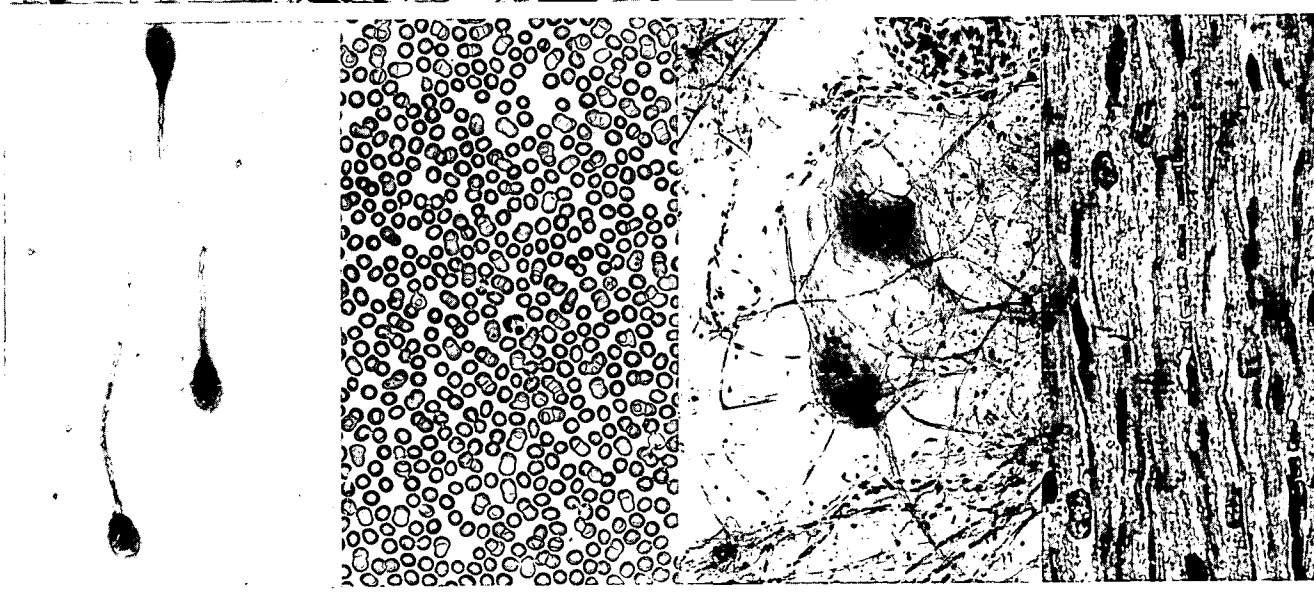
*Piet Hein is a Scandinavian poet who often served as a "mental Ping-Pong partner" for the famous chemist Niels Bohr.

Contents

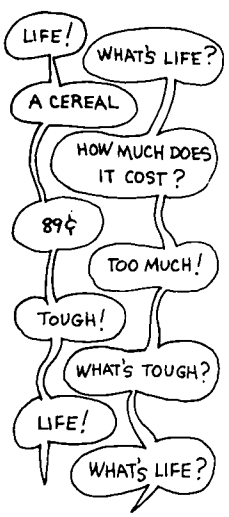
Considering Life Processes

What is a living thing? At first this might seem to be an easy question to answer. But as a matter of fact, it's a puzzling question to scientists. Our present definition of "living" matter is based on the special types of activities, the unique properties that we have learned to associate with living systems. But what is responsible for these types of activities, for these unique properties? And how can they be explained?

Biochemical studies of cells, such as sperm, blood, nerve, and muscle tissue, that carry out life processes help us to understand the stages and activities we recognize as being associated with life.



CAROLINA BIOLOGICAL SUPPLY COMPANY



B-1 The Chemistry of Life

If we consider the properties of a solid chunk of ice or a solution of sodium chloride in water, we know that their properties can be readily explained in terms of chemical theories and laws. Can we use these same theories and laws to examine living things? Are there specific molecules responsible for the properties we have learned to associate with living things?

For a moment consider living systems—bacteria, plants, animals, or even yourself. There are many similarities among living things. For example, all have the ability to reproduce. Are certain types of molecules involved in reproduction? If so, how do these molecules perform this function?

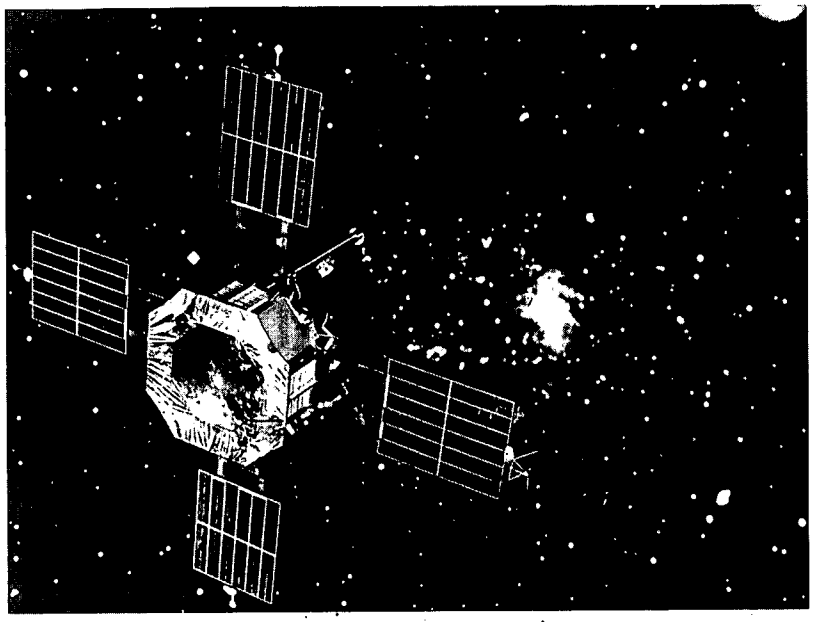
Consider another aspect of living systems. All living things have a life cycle. In this cycle we see a regular, predictable pattern. Stages such as birth, growth, development, maturity, old age, and death appear in a well-defined order. Each stage appears to be specifically controlled. Are there specific molecules that regulate events?

A most impressive property of biological systems is their large variety of activities. Eating, sleeping, moving, talking, thinking—these are activities familiar to each of us. Are there unique chemical reactions behind each of these activities? If so, what turns these reactions on? How are they turned off? What are the molecules involved in these reactions?

The questions we have asked are just a few of the many questions that biochemists seek to answer as they investigate the chemistry of life. Answers to such questions are not always easy to obtain because biological systems are extremely complex. Partly because of this complexity, scientists believed until the early nineteenth century that compounds found in living systems had their own special properties. These early scientists believed that compounds in living systems were entirely different from compounds found in nonliving systems and that only living things could make *biomolecules*. (The prefix *bio* means "life.") Many believed that biomolecules were subject to a set of rules that were different from the rules for other molecules.

Of course, it is not true that biomolecules are subject to a different set of rules. Scientists have been able to *synthesize* (to make) many biomolecules in the laboratory without the aid of a living system. We now know that the same relatively simple chemical principles govern all molecules, whether in living systems or nonliving systems.

Once this was clear, the science of *biochemistry* began to develop rapidly. Using relatively simple chemical principles, biochemists examined the structure and reactivity of many biomolecules. A



Our insatiable curiosity about life elsewhere in the universe triggered the Mariner space flights, which, since 1965, have measured atmospheric densities and temperatures of other planets. Findings to date suggest that life as we know it does not exist on Venus, with surface temperatures of 540°C, or on Mars, which has polar caps of frozen carbon dioxide and is veiled in intense ultraviolet radiation. As we investigate life on earth, our search for the existence of life on other planets will still continue.

large body of information on biomolecules is now available. This information is extremely useful. In many instances the established facts and principles allow biochemists to understand the very functions of biomolecules in living systems.

In this module we will look at some of the biomolecules that are known to function in specific biological roles. We will examine some of their properties and observe their reactivity. As we do this, we will try to understand how such information can help explain the specific biological functions of these molecules.

B-2 Functions of Biomolecules

The molecules involved in biological processes are known as *biomolecules*. Biomolecules can be grouped according to their functions in living systems—that is, according to their roles in living systems. These molecules play at least one of six roles. The six roles are listed in Table 1. As you will note, some biomolecules have a role to play in reproduction. Others are involved in control and regulation. Still others function as nutrients, as catalysts, as structural components, or as sources of energy.

You probably recognize some of these biomolecules even though you may not be familiar with the "official" names used in the table. In identifying biomolecules, the biochemist often subdivides biological compounds into general classes. Although there are others, we will deal primarily with four major classes: (1) *carbohydrates*, (2) *nucleic acids*, (3) *proteins* (and *amino acids*), and (4) *lipids* (*fats*).

It would be nice if one class of biomolecules, such as proteins and amino acids, functioned in only one biological role. Unfortunately, this is not the case. Some proteins have structural roles. The protein found in cell membranes and the proteins in your skin, fingernails, and hair are examples. Other proteins are hormones and function in controlling or regulatory roles. One such hormone is called *insulin*. People who do not have enough of this protein hormone suffer from a disease known as *diabetes*. Still other proteins function as *catalysts* in living things. As you may already know, catalysts are substances that make reactions go faster. Proteins that function as catalysts are so important that they have been given a special name. We call these protein catalysts *enzymes*.

TABLE 1: SOME BIOMOLECULES AND THEIR BIOLOGICAL ROLES

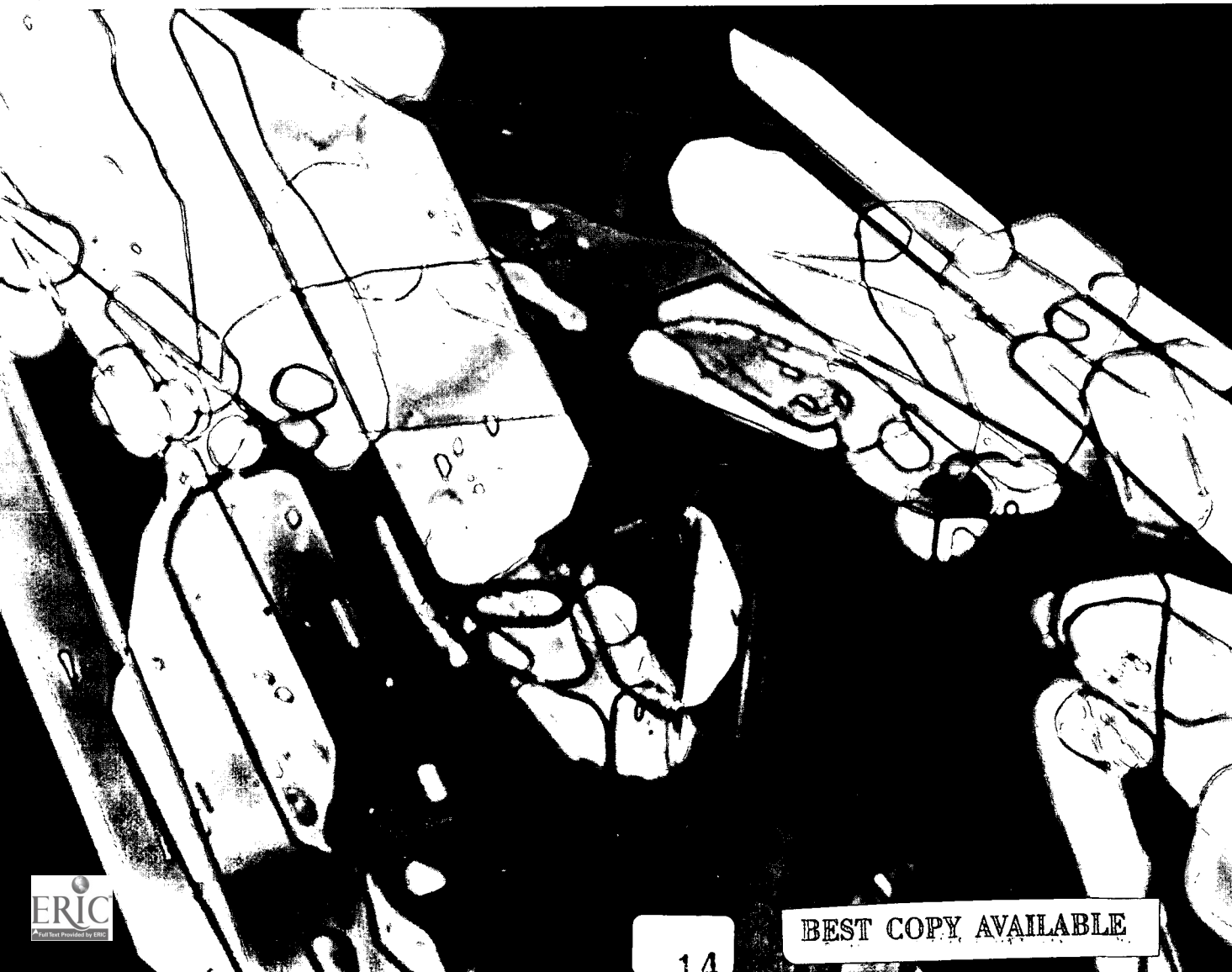
Biomolecule	Biochemical Class	Role of Biomolecule
Hair protein Skin protein Membrane protein Membrane lipid Cellulose	Protein Protein Protein Lipid Carbohydrate	*As structural components:* For example, these are found in hair, bone, skin, cell walls of plants, and cell membranes.
DNA* RNA**	Nucleic acid Nucleic acid	*In reproduction and information storage:* These molecules store and transfer hereditary (genetic) information.
Insulin (a hormone) Testosterone (a hormone) Estradiol (a hormone)	Protein Lipid Lipid	*In control and regulation:* Insulin controls the concentration of glucose in the blood. Testosterone is a male sex hormone, and estradiol is a female sex hormone.
Succinate dehydrogenase (enzyme) Trypsin (enzyme) Lipase (enzyme	Protein Protein Protein	*In catalysis:* Enzymes increase the rate of biochemical reactions.
Ascorbic acid (vitamin C) Retinal (vitamin A)	Carbohydrate Lipid	*As essential nutrients:* Many organisms cannot synthesize all the compounds they need. These necessary compounds must be obtained in the diet.
Starch Glucose Fats and oils ATP† (the key energy compound)	Carbohydrate Carbohydrate Lipid Related to nucleic acids	*As energy sources:* The activities of living things require energy. These are examples of compounds that can supply and store this energy.

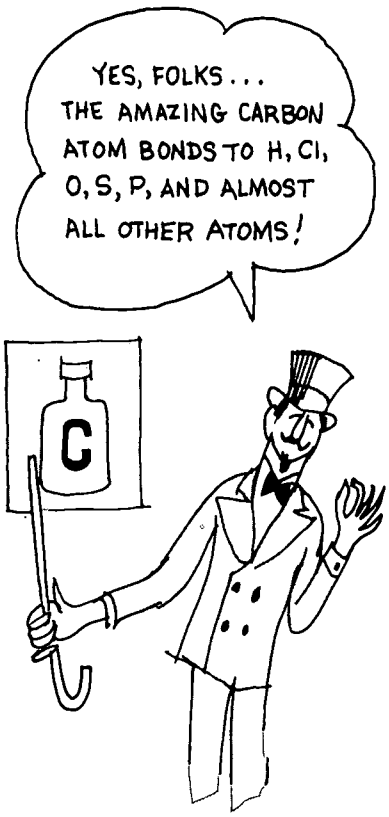
*DNA = deoxyribonucleic acid
** RNA = ribonucleic acid
†ATP = adenosine triphosphate

Understanding the Structure of Biomolecules

Proteins have many biological roles. This is also true for other classes of biomolecules, although we cannot differentiate the classes of molecules solely by examining their biological roles. Then how do we differentiate between these classes of biomolecules? What are proteins and amino acids? How do we know that proteins are different from lipids and carbohydrates? In order to explain the differences between biomolecules, we must understand their structures.

Highly magnified crystals of an amino acid used in creating a new antibiotic by chemically modifying a natural molecule. Without understanding the structure of biomolecules this would have been impossible.





B-3 The Amazing Carbon Atom

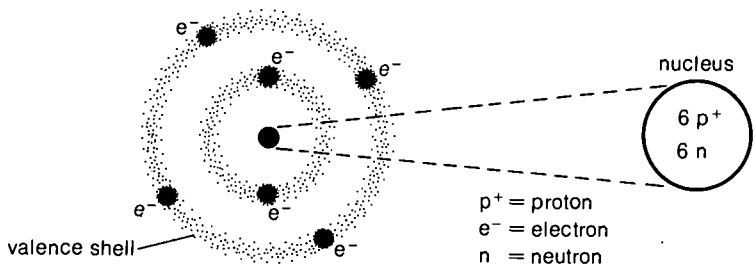
In the most general sense, proteins and amino acids, lipids, and carbohydrates are all organic compounds. This simply means that they all contain the element carbon. Almost all organic compounds also contain hydrogen. In addition, many of the biological carbon compounds contain oxygen, nitrogen, phosphorus, and sulfur.

No one knows exactly how many different carbon compounds exist. It is estimated that millions of different carbon compounds have been studied; thousands of new carbon compounds are added to the list each year. If each carbon compound is different, each compound must have a unique structure. What do we know about the chemistry of the carbon atom? How can we explain the formation of so many different structures?

Carbon has four electrons in its outer electron shell. Carbon atoms use these electrons to form bonds with other atoms. Since this outer electron shell is often called the *valence shell,* the electrons involved in the bonding are often referred to as *valence electrons.* We also know that *carbon is one of the elements that will form stable compounds when it is sharing a total of eight valence electrons.* This means that every carbon atom must obtain a share of four additional electrons from other atoms before it will form a stable compound.

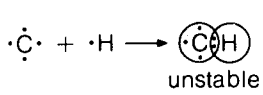
A schematic picture of a carbon atom is shown below. Can you draw a similar picture for a hydrogen atom? Each hydrogen atom has a single electron. *When hydrogen forms stable compounds, it has a total of two electrons in its valence shell.* Each hydrogen atom has one valence electron, and each carbon atom has four valence electrons. Considering this, would you predict the formation of a stable compound if one hydrogen atom were bonded to one carbon atom?

SCHEMATIC CARBON ATOM



A carbon atom bonded to only one hydrogen atom has a total of only five valence electrons. It has four from its valence shell and one from the hydrogen valence shell. We have already said

that carbon must have a share of eight valence electrons before it will form a stable compound. Therefore, this combination does not form a stable compound.



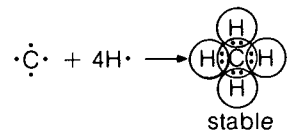
unstable

e^- shared by each H = 2

e^- shared by C = 5

Each dot represents a valence electron (e^-).

On the other hand, if four hydrogen atoms "combined" with one carbon atom, we would predict the formation of a stable covalent compound.

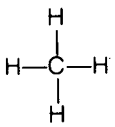


stable

e^- shared by each H = 2

e^- shared by C = 8

The dot structures that you see used here are called *Lewis dot structures.* In such a structure, two dots between any pair of atoms represent a pair of electrons in a covalent bond. Chemists often prefer simply to draw a line between two atoms. The line represents each pair of shared electrons. The stable compound represented by the combination of one carbon atom and four hydrogen atoms can be shown either way.

METHANE	CH_4		
	molecular formula	Lewis dot structure	line structure

This compound has the molecular formula CH_4 and is called *methane*. It is one of the simplest organic compounds. In constructing this simple compound, we observed three of the basic rules that chemists use in showing the structure of covalent compounds:

1. In a covalent compound, each bond is formed between two atoms by sharing two valence electrons.
2. Each carbon atom forms four bonds in all of its covalent compounds.
3. Each hydrogen atom forms one bond in all of its covalent compounds.

EXERCISE

Using the rules that we have just discussed, draw both the Lewis dot and the line structures for ethane. This compound has the molecular formula C_2H_6.

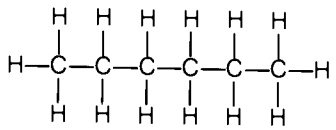
TIME MACHINE

1911	Willis H. Carrier invents air conditioning.
1912	Juliette Gordon Low organizes the Girl Scouts.
1913	The first crossword puzzle is published.
1916	**Gilbert N. Lewis identifies the valence, or chemical bond, as a pair of electrons shared by two atoms that serves to hold the atoms together. He illustrates this with the electron dot system.**
1918	Regular airmail service begins, between Washington, D.C., and New York.
1919	The first scheduled air-passenger service—between London and Paris—begins.
1920	Babe Ruth, age 25, is sold by Boston Red Sox to New York Yankees for $125,000.

As you drew the structures for the compound ethane, you no doubt noticed that the rules forced you to combine these atoms in a very special way. You had to show one bond between the two carbon atoms.

Consider the line structure for the molecule known as *hexane*:

HEXANE C_6H_{14}



Hexane has the molecular formula C_6H_{14}. As you can see, carbon atoms have the ability to form bonds with other carbon atoms. This is one of the reasons there are so many carbon compounds.

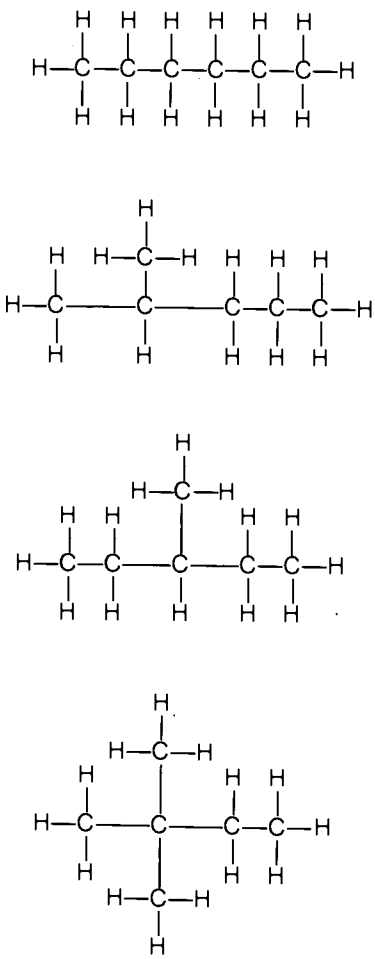
The compound hexane is not the only organic compound with the molecular formula C_6H_{14}. There are three other compounds with this formula. This is because the same six carbon atoms can be arranged in different ways. For example, in combining carbon atoms, one carbon atom can be bonded to either one, two, three, or four other carbon atoms. The different ways of combining the same number of carbon atoms is another reason for the large number of carbon compounds. Compounds that have the same molecular formula but different structural patterns are called *isomers*.

The compounds that we have discussed so far have only hydrogen and carbon atoms. Such compounds belong to a large group called *hydrocarbons*. Can you see where the name came from? The hydrocarbons are relatively unreactive compounds. This simply means that they undergo few interesting chemical reactions when compared with other compounds.

If we introduce an element in addition to hydrogen into a carbon compound, we can drastically change the compound's reactivity. Because of this, we speak of *reactive groups* when elements other than hydrogen are present in a carbon compound. When the same reactive group is present in different compounds, we note that the different compounds "function" in similar ways. For this reason, these reactive groups are called *functional groups*.

ISOMERS OF HEXANE

Molecular formula: C_6H_{14}



The atoms are arranged in different ways in each of the four isomers of C_6H_{14}. Each isomer contains six carbon atoms, but the structures differ.

B-4 Functional Groups: Key to Reactivity

Other than hydrogen, oxygen is the most common element found in carbon compounds. What happens when oxygen is covalently bonded to other elements? Oxygen has six electrons in its valence shell. It must share two additional electrons to form a stable covalent compound. This is exactly what happens in the water

molecule. Two hydrogen atoms share their valence electrons with an oxygen atom to form a stable covalent compound.

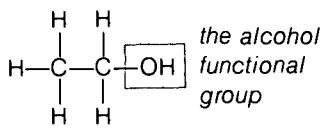
Now we have a fourth rule to add to the three established in the preceding section.

1. In a covalent compound, each bond is formed between two atoms by sharing two valence electrons.
2. Each carbon atom forms four bonds in all of its covalent compounds.
3. Each hydrogen atom forms one bond in all of its covalent compounds.
4. *Each oxygen atom forms two bonds in all of its covalent compounds.*

Using these four rules, we can show the structures of a large number of carbon compounds. First, let us look at some relatively simple molecules and three common functional groups.

The active substance in alcoholic beverages is ethyl alcohol (also known as *ethanol*). In addition to being a potent drug, it is an important industrial chemical. As the name implies, ethyl alcohol contains the *alcohol functional group* (—OH) and has the molecular formula C_2H_6O. Using the rules we have established, we have drawn the following structure for this compound:

ETHYL ALCOHOL C_2H_5OH



Note that the reactive group is an —OH group. This is the alcohol functional group. When we wish to emphasize that a molecule contains an alcohol group, the —OH is written separately in the molecular formula. For example, ethyl alcohol can be shown as C_2H_5OH. Any molecule that contains an alcohol group will undergo many of the same reactions as ethyl alcohol.

Rule 4 states that each oxygen atom forms two bonds in all of its covalent compounds. When an oxygen atom forms bonds with only one carbon atom, we have a *carbonyl group* (—CO). For this to occur, the oxygen atom and the carbon atom in the carbonyl group, must share four electrons. When two atoms share four electrons, we have a double bond. We show a double bond by drawing two lines between the atoms.

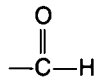
The *carbonyl group*

COMMON FUNCTIONAL GROUPS

alcohol group

—OH

aldehyde group

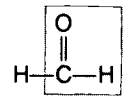


ketone group

Carbonyl groups are very common in biological compounds, but we often call them by other names. The names we use depend on the groups attached to the *carbon atom* of the carbonyl group. Some are called *aldehydes* and others *ketones*. Later in the module we will discuss still others called *carboxylic acids, esters,* or *amides.* Since these compounds all contain the carbonyl group, you might expect them to undergo many of the same reactions. Sometimes they do. However, they often undergo very different reactions. For this reason, it is useful to give them different names.

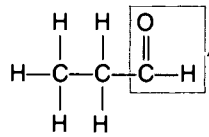
The compounds in the following illustration are *aldehydes* (—CHO). They are so named because there is a hydrogen atom attached directly to the carbon atom of the carbonyl group in each compound.

FORMALDEHYDE HCHO



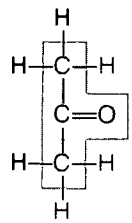
the aldehyde functional group

PROPIONALDEHYDE C_2H_5CHO

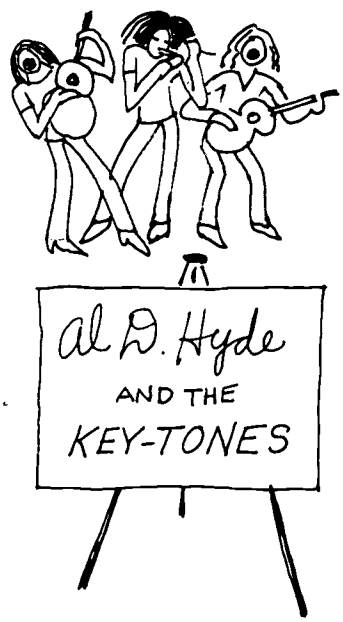


If we slightly rearrange the way in which the atoms are combined in a molecule of *propionaldehyde*, we get a compound with the following structure:

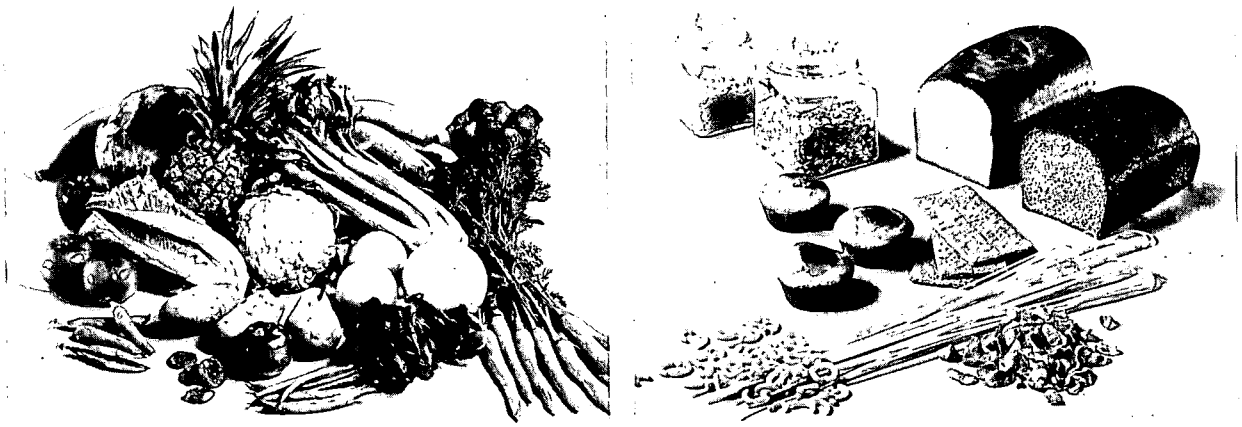
ACETONE $(CH_3)_2CO$



the ketone functional group



Having done this, we no longer have an aldehyde; we have a ketone. Its name is *acetone*. We emphasize its unique structure by writing the molecular formula as $(CH_3)_2CO$. Acetone is a ketone because there are two carbon atoms attached to the carbon atom of the carbonyl group. Compare the structures of acetone and propionaldehyde. You can see how ketones and aldehydes differ. Notice that the molecular formulas are written to emphasize this difference. The molecular formula for both compounds could be written C_3H_6O. When the formula is written this way, you can easily see that acetone and propionaldehyde are isomers because they have the same molecular formula but different structures.

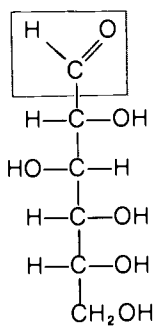


About two-thirds of our food intake is made up of carbohydrates. Two of the basic food groups are shown here—vegetables and fruits and grains and cereals. These foods are high in carbohydrates.

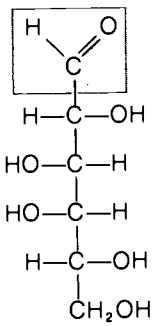
Thus far we have restricted our discussion to molecules that contain a single functional group. However, most biomolecules have more than one functional group. *Carbohydrates* are an example. Many of the common small carbohydrate molecules have identical molecular formulas. They are isomers of each other. For example, a large number of different carbohydrates have the formula $C_6H_{12}O_6$. As an exercise, try to draw structures for compounds with this formula. How many different structures can you come up with? How do you know they are different?

ALDEHYDE-CONTAINING SUGARS

GLUCOSE



GALACTOSE



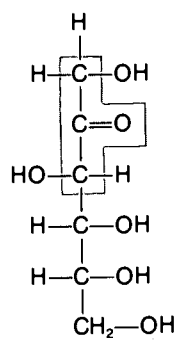
B-5 Carbohydrates

The term *carbohydrates* arose from early studies of common sugars such as *glucose* and *fructose*, both of which have the molecular formula $C_6H_{12}O_6$. They fit easily into a general formula that suggested to some that they were hydrates of carbon: $C_6(H_2O)_6$. Although we now know that they are not hydrates of carbon, the name has persisted. Some people confuse the term carbohydrate with hydrocarbon. It should be easy to see that they are not the same. Why?

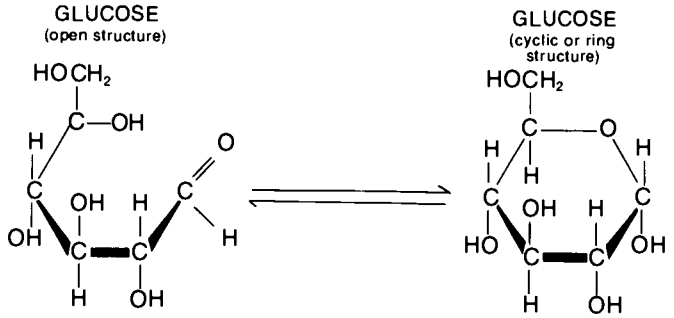
The structures in the margin are simple carbohydrates and are called *monosaccharides*. Note that they all contain the *alcohol functional group*. In fact, each molecule has five of the groups. Glucose and galactose also contain an *aldehyde group*. Fructose is different from the others. It contains a *ketone group*.

KETONE-CONTAINING SUGAR

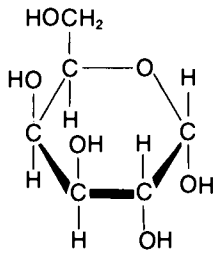
FRUCTOSE



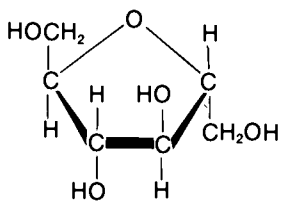
Aldehyde and ketone groups are very reactive. One of the common reactions of these groups is illustrated in the following figure.



GALACTOSE
(cyclic structure)



FRUCTOSE
(cyclic structure)



An alcohol group can react with an aldehyde group in the same glucose molecule. A similar reaction occurs between an alcohol group and the ketone group in fructose. These reactions are quite common, especially when a five- or six-membered ring can be formed in the product. In fact, most of the glucose, galactose, and fructose found in biological systems exist in a cyclic (ring) form.

Instead of looking at these structures from the top of the ring, we want to look at them from the edge of the ring. The darker, heavier lines represent the edge of the ring closest to you. The oxygen atom in the ring is toward the back. Some of the alcohol groups attached to the carbon atoms in the ring are above the ring and others are below. Try to locate these. If you compare the structures of glucose and galactose in this way, you can see how they are different. One alcohol group that is below the ring in glucose is above the ring in galactose. This may seem to be a very small difference, but the next experiment will show how important this difference is.

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miniexperiment

This symbol represents three of the common hazards in a chemistry laboratory—flame, fumes, and explosion. It will appear with certain experiments in this module to alert you to potential hazards.

B-6 How Sweet It Is

Before beginning this first experiment in the module, thoroughly acquaint yourself with the safety precautions outlined in Appendix I.

In this miniexperiment you will taste several different sugars. You will rate each of the compounds on the basis of its sweetness. *Best results are obtained if you rinse your mouth out with some water before you taste each new compound.*

Record your results in a table similar to the one that follows.

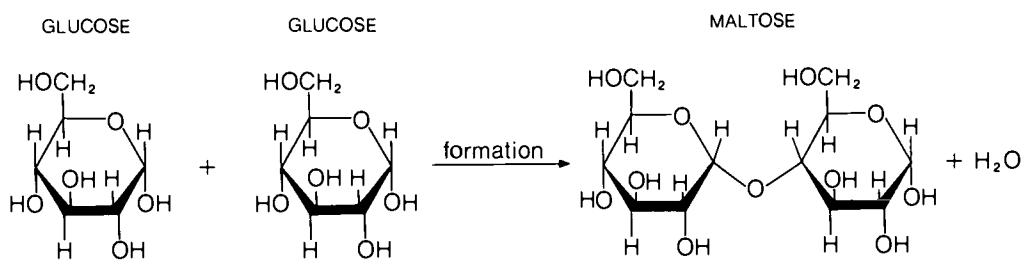
Caution: *This experiment is unusual. Tasting chemicals can be extremely dangerous and should never be done unless specifically instructed.*

Compound	Molecular formula	Relative Sweetness		
		very sweet	medium sweet	not sweet
glucose	$C_6H_{12}O_6$			
galactose	$C_6H_{12}O_6$			
fructose	$C_6H_{12}O_6$			
sucrose	$C_{12}H_{22}O_{11}$			
maltose	$C_{12}H_{22}O_{11}$			
lactose	$C_{12}H_{22}O_{11}$			
others . . .				

Questions:

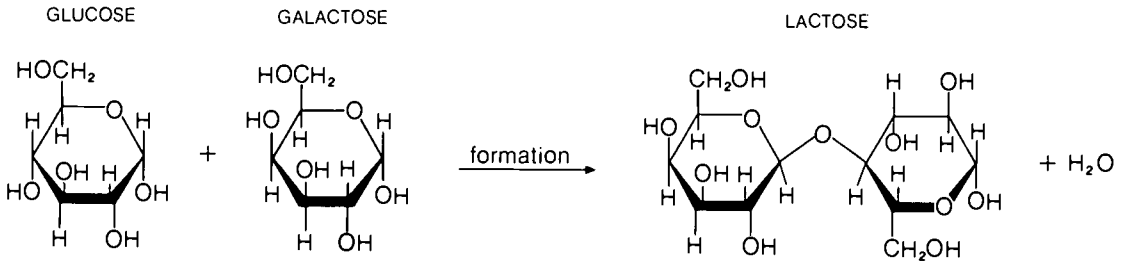
1. Which compound was the sweetest?
2. Which was least sweet?
3. Is there a difference between glucose and galactose?

One compound that you tasted is called *maltose*. Its structure is shown in the following reaction. Notice that the carbon atoms in the ring are not shown in the diagram. We will use these simpler structures for the rest of the module.

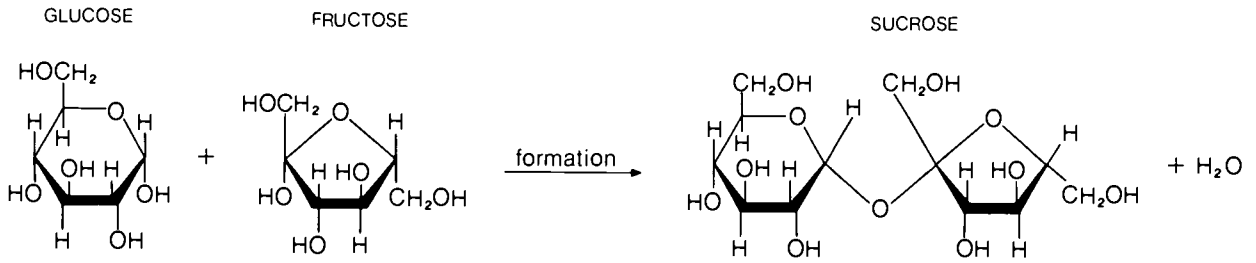


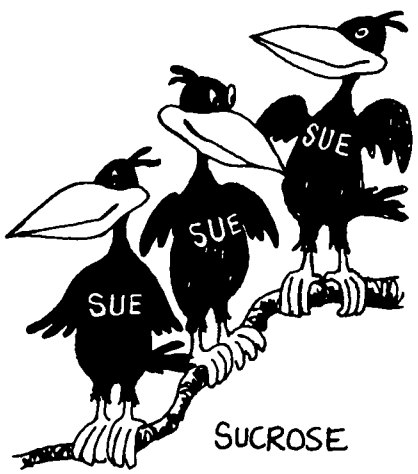
You can see that maltose looks like two glucose molecules connected by a common oxygen atom. In biological systems, two molecules of glucose can react with each other. In the process, a molecule of water is split out and a molecule of maltose is formed.

In a similar manner, one glucose molecule can combine with a molecule of galactose to form the compound *lactose*.



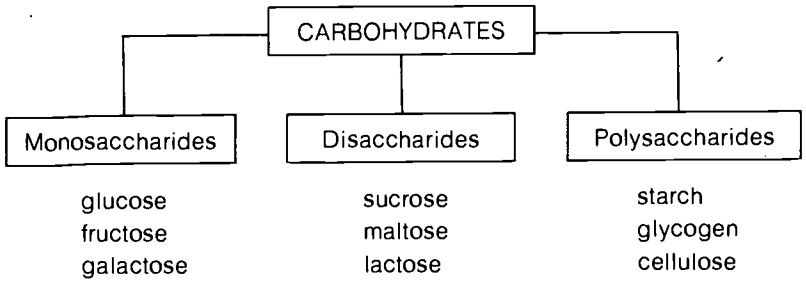
Still another sugar is formed when a molecule of glucose is covalently bonded to a molecule of fructose. You are familiar with this compound. It is called *sucrose*, but is better known as just plain table sugar.





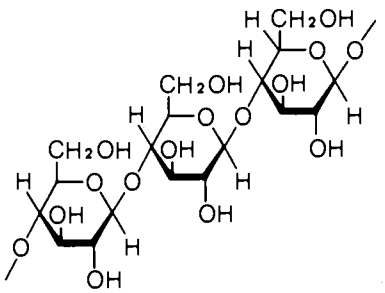
All carbohydrates mentioned in this section are called *saccharides*. In fact, the name *saccharide* literally means "sugar." Glucose, galactose, and fructose are called *mono*saccharides. Sucrose, maltose, and lactose are called *di*saccharides because they consist of two simple sugar molecules linked together.

The reaction of one glucose molecule with another glucose molecule is important in biological systems. Glucose is used by your body when energy is needed. If extra glucose is present, the glucose is stored. But it is not stored as a monosaccharide, nor is it usually stored as a disaccharide. Instead, enzymes convert the extra glucose into larger molecules called *polysaccharides*. (The prefix *poly* means "many.") Instead of stopping with the formation of the disaccharide maltose, most living systems continue to add glucose molecules together until very large macromolecules are synthesized. In plants, this carbohydrate macromolecule is called *starch*. In animals, the glucose is stored as the polysaccharide called *glycogen*. Both of these carbohydrates are large macromolecules composed of many, many glucose units.

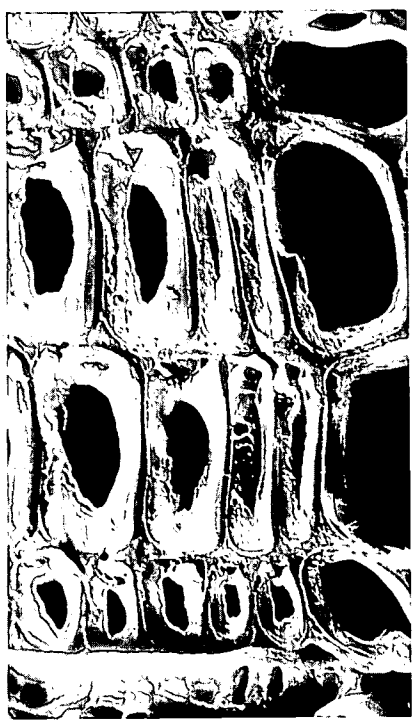


The relationship among the compounds that we have discussed is illustrated in the preceding diagram. These are a few of the more common biological compounds that are classified as carbohydrates. Cellulose is a carbohydrate that is used primarily as a structural biomolecule. This macromolecule is composed of about two thousand to three thousand glucose units. Cotton, linen, and paper are good examples of relatively pure cellulose. The stringy nature of celery is partly the result of its cellulose content. Even though cellulose is a polysaccharide composed of glucose units, many animals, including human beings, cannot use it as a source of energy. This is because we lack the specific enzymes necessary to convert cellulose back into glucose units.

CELLULOSE



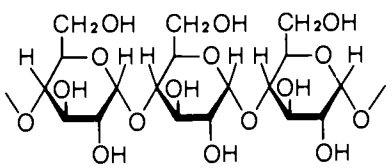
A micrograph of the cellulose cell walls in plant tissue—wood (magnified 675 times). Would you agree that cellulose is a major constituent of this plant?



B-7 Carbohydrates as Energy Compounds

Starch and glycogen are similar to cellulose. They are all composed of many glucose units. But starch and glycogen differ from cellulose in one important way. The glucose units in starch and glycogen are joined differently from the way glucose units in cellulose are joined. For this reason, the enzymes present in your body can convert starch and glycogen back into glucose units. Thus, these carbohydrates are digested by human beings, whereas cellulose is not.

STARCH



When you digest a carbohydrate, such as potato starch, an enzyme present in saliva converts this polysaccharide into maltose, a disaccharide. The maltose is taken through the stomach and into the small intestine. In the small intestine another enzyme

Plant cells having cellulose walls and containing starch grains (magnified 1000 times).

splits the maltose molecule into glucose units. The glucose can then be absorbed into the bloodstream and carried to the parts of the body that require energy.

Inside living cells, glucose is rapidly converted to carbon dioxide and water through a long series of enzymatic reactions. In the process a large amount of energy is also released. Much of the energy is stored in the cell by reactions which synthesize *adenosine triphosophate* (*ATP*). As glucose is broken down, ATP is formed. This ATP is directly involved in all body reactions that require energy input. Your body cannot store large amounts of ATP. Thus, as ATP is used up in reactions, glucose or some other source of energy must be broken down to replenish the supplies of ATP. We will have more to say about ATP later.

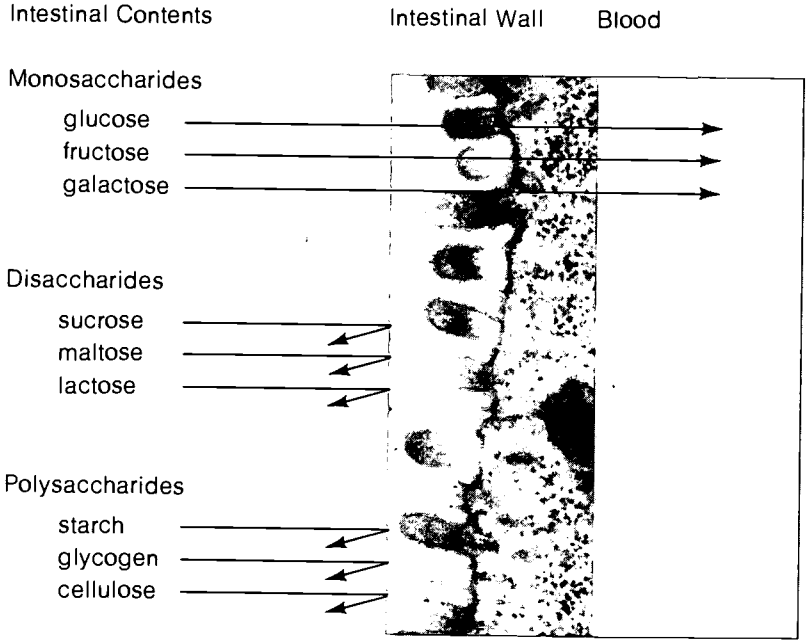
If there were no way to store carbohydrates, we would be eating every minute of the day. Fortunately, when excess glucose is present in living systems, it is converted into its storage form. In plant systems this is the polysaccharide starch, and in animal systems the polysaccharide is glycogen. Thus, when food is not available and large amounts of energy are required, the glycogen that we have stored in our bodies can be converted back into glucose. Glucose is an important carbohydrate. But what about the other monosaccharides and disaccharides? What is their fate in living systems?

When you take in sucrose or lactose as food, enzymes in the intestine convert them into their component monosaccharides. Thus, all carbohydrates that are ingested are eventually converted

TIME MACHINE

1853	Matthew Perry leads a mission to open Japan to foreign trade.
1854	Henry David Thoreau writes *Walden*.
1855	**Claude Bernard proves that glycogen from the liver is converted to blood glucose.**
1856	James Kelly and Jack Smith fight longest bareknuckle bout—186 rounds.
1857	U.S. Supreme Court's Dred Scott decision states slave is not free when taken into a free state.
1858	Wilhelm Busch publishes "Max und Moritz," the first comic strip.
1859	Charles Darwin's *On the Origin of Species by Natural Selection* is published.
1860	Abraham Lincoln is elected 16th president of the United States.

SELECTIVE ABSORPTION OF CARBOHYDRATES BY THE INTESTINE



into monosaccharides. These monosaccharides are important because they can be readily absorbed through the intestinal wall, passed into the bloodstream, and utilized as energy compounds. Disaccharides and larger carbohydrates cannot be absorbed by the intestine and remain in the intestinal contents. If a person cannot digest the larger carbohydrates, they will not be absorbed and utilized by the cells.

In most cases, a different enzyme is needed to break apart each different disaccharide in the intestine. Apparently, everyone has the enzyme necessary to convert sucrose into glucose and fructose. However, some people do not have the enzyme needed to convert lactose (milk sugar) into glucose and galactose; instead lactose remains in the intestine and causes cramps and diarrhea. This syndrome is called "lactose intolerance." Since lactose is found primarily in milk, people who lack this enzyme show these symptoms only when they drink milk or consume other dairy products that contain lactose. This condition is rarely found in children but occurs frequently in adults of non-European descent. As infants they have the lactose-splitting enzyme, but they lose it as they grow older.

A scientist studies the properties of the biomolecules in milk that make it more digestible. This sample of milk has been run through a column of immobilized enzymes to hydrolyze the lactose.

B-8 Lipids: Another Source of Energy

Theoretically, any compound that can be converted to water and carbon dioxide can also be used to supply energy. However, not all compounds can be stored for this purpose. We have already seen how glucose can be stored as the polysaccharide glycogen in animal cells or as starch in plant cells.

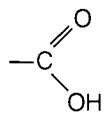
The lipids, which we usually think of as fats and oils, are an important source of heat and energy. Do you know which of these lipids are saturated, which are unsaturated, and which are polyunsaturated? Read on, and then take a closer look at the labels on the grocer's shelves.

Another class of biomolecules can also serve as energy storage compounds. These compounds are commonly called fats or oils. What are fats and oils? The chemist classifies them as *lipids*. But what are lipids?

One example of a relatively simple lipid is *palmitic acid*. It was given this name because it was isolated in large quantities from the fruit of palm trees. Now we know that it is found in many organisms.

Palmitic acid has several important features. First, notice that most of the molecule is simply carbon and hydrogen. The carbon atom at one end of the molecule is bonded to two oxygen atoms. Except for this carbon atom, the rest of the molecule resembles a long hydrocarbon. Do you remember what a hydrocarbon is? The most important thing about a hydrocarbon is that it contains no functional groups. Some people have said that palmitic acid looks something like a caterpillar—a head with a long hydrocarbon tail.

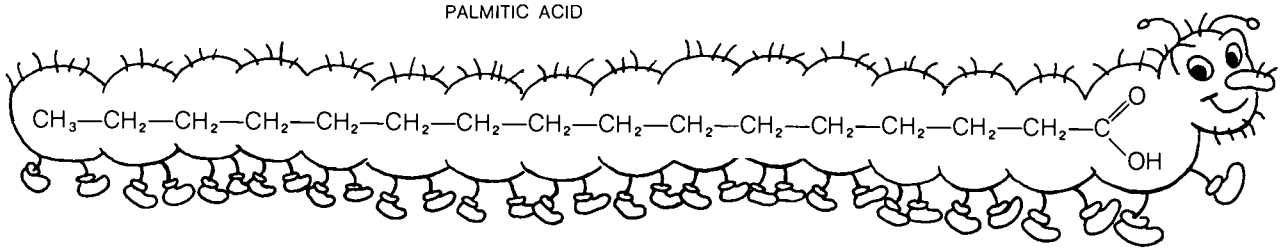
What about the oxygen atoms in the palmitic acid molecule? We have seen carbon atoms bonded to oxygen atoms before when we talked about the carbonyl groups in aldehydes and ketones. But this compound is different from an aldehyde or a ketone because there is an —OH group attached directly to the carbon atom of the carbonyl. This —OH group is not an alcohol group because it is attached to the carbonyl. Instead, we speak of a new functional group. The group



is called the *carboxylic acid group* (or *carboxyl group*). As you might expect, this group undergoes very different reactions from those of the aldehyde group or the ketone group. We can emphasize the presence of this group in palmitic acid by writing the molecular formula as $C_{15}H_{31}COOH$.

Palmitic acid is a member of a large group of lipids called *fatty acids*. All of these compounds have the carboxylic acid functional group, and all are found in fats. Other fatty acids differ from

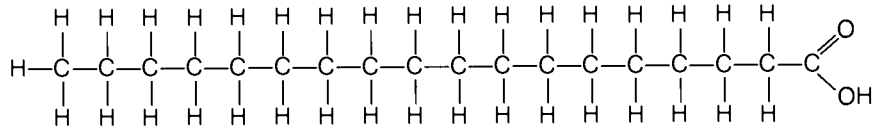
PALMITIC ACID



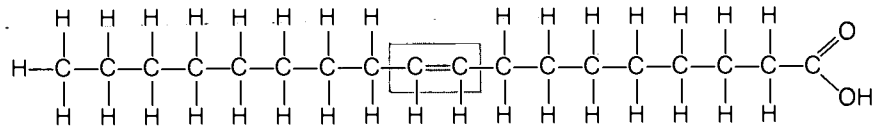
palmitic acid primarily in two ways: (1) the hydrocarbon tail can be longer or shorter; and (2) double bonds can be present in the hydrocarbon end of the molecule.

Compare the structures of three compounds: *stearic acid, oleic acid,* and *linolenic acid,* illustrated in the following diagram. A quick look at the molecular formulas indicates that they all have the same number of carbon atoms. Also, they all have a carboxylic acid functional group. Furthermore, they are all found in lipids. Thus, they are all fatty acids.

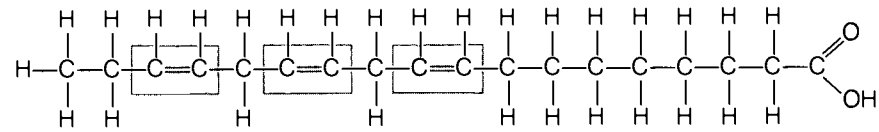
STEARIC ACID ($C_{17}H_{35}COOH$)



OLEIC ACID ($C_{17}H_{33}COOH$)



LINOLENIC ACID ($C_{17}H_{29}COOH$)

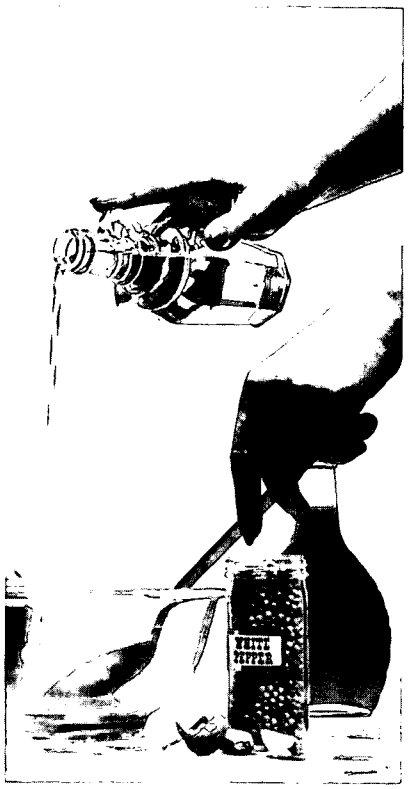


Note that all of these fatty acids have a carboxyl group and that two of them have carbon-carbon double bonds.

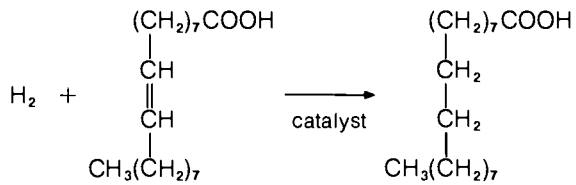
The only difference among these three fatty acids is the number of hydrogen atoms in the compounds. Compared with stearic acid, oleic acid has two fewer hydrogen atoms and linolenic acid has six fewer hydrogen atoms.

The valence requirements of carbon atoms must be satisfied in other ways when there are not enough hydrogen atoms in a compound. Thus, carbon atoms with insufficient hydrogen atoms form *double bonds* with each other. For each two hydrogen atoms that are missing, another bond must be formed between two carbon atoms. Thus, oleic acid, which has two hydrogen atoms less than stearic acid, has one double bond. Linolenic acid has three double bonds. Can you see why?

The ability of one carbon atom to form double bonds with other carbon atoms is another reason for the amazing variety of carbon compounds. The presence of double bonds between carbon atoms in the hydrocarbon tails makes these molecules somewhat more reactive. For example, a common reaction of compounds that contain the C=C structure is called *hydrogenation* (the addition



of hydrogen). Oleic acid undergoes this reaction and is converted to stearic acid. Stearic acid does not undergo hydrogenation because it already has as many hydrogen atoms as the carbon atoms can accept. We say that stearic acid is a *saturated* compound. It can accept no more hydrogen atoms.



On the other hand, oleic acid can add two more hydrogen atoms in covalent bonds. We say that oleic acid is an *unsaturated* compound. Likewise, linolenic acid is also unsaturated and can be converted into the saturated fatty acid, stearic acid, by the hydrogenation reaction. Because linolenic acid has more than one double bond, it is called a *polyunsaturated* fatty acid.

You have probably heard of or have seen food products which claim to contain polyunsaturated fatty acids. During the 1960s, biochemists became quite concerned about the influence of dietary saturated and unsaturated fatty acids on health. Diets consisting of animal fats, such as butter, beef fat, and lard, which are high in saturated fatty acids and low in polyunsaturated fatty acids, are implicated in heart disease. On the other hand, most plant fats contain unsaturated and polyunsaturated fatty acids, allowing heart patients to eat them. For this reason, consumers began switching from products that are low in polyunsaturated fatty acids to foods that contain larger quantities of polyunsaturated fats.

Prior to 1960, polyunsaturated fats were considered quite undesirable. Products that contain fatty acids with reactive double bonds undergo "oxidation" reactions with oxygen in the air. These reactions cause the products to have bad tastes and odors. Also, products that contain large quantities of polyunsaturated fatty acids are liquids (oils) at room temperature, unlike saturated fat products such as butter. A major use of fats is in spreads. Butter left at room temperature spreads easily on bread, but liquid polyunsaturated fats simply ooze through the bread.

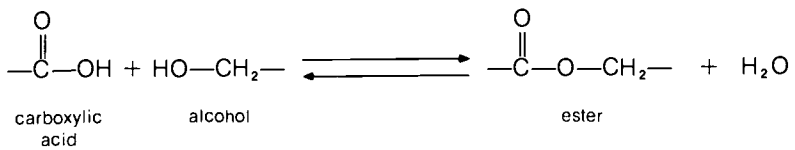
Since many vegetable fats are much less expensive than animal fats, the food industry went to great pains to perfect a vegetable product that would have consumer appeal. Thus, margarine was developed. Margarine, in most cases, is made from vegetable oil that has been partially hydrogenated to give it spreading qualities.

The fatty acids that we have been talking about do not actually exist as fatty acids in most fats and oils. The carboxylic acid

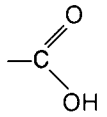
TIME MACHINE

1867	United States buys Alaska from Russia for $7,200,000.
1868	Louisa May Alcott writes *Little Women*.
1869	D. I. Mendeleev theorizes that certain properties of the elements are a periodic function of their atomic weights.
1870	**The French chemist Hippolyte Mege-Mouries develops margarine and wins a prize bestowed by Napoleon III.**
1871	James McNeill Whistler paints *Arrangement in Grey and Black*, commonly known as "Whistler's Mother."
1871	Journalist Henry M. Stanley finds Dr. David Livingstone at Ujiji in Tanzania.

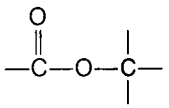
group is usually present as another functional group called an *ester*, which is the result of a reaction between a carboxylic acid and an alcohol:



carboxylic acid group (carboxyl group)



ester functional group

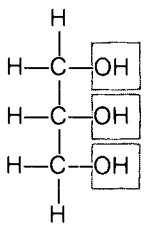


The alcohol in fats is known as *glycerol* (sometimes called *glycerin*). Glycerol is a compound that has three alcohol groups.

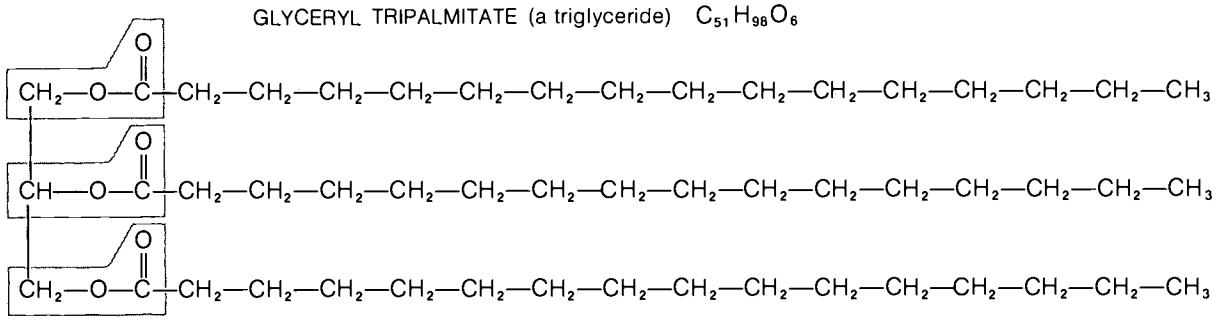
One molecule of glycerol can react with three molecules of fatty acid to form a new compound that is called a *triglyceride*. When people speak of fats and oils, they are actually talking about triglycerides.

The most striking feature of this triglyceride is the large, hydrocarbonlike portion of the molecule. (We will return to this point in the next experiment.) Also, the molecule no longer has carboxylic acid or alcohol functional groups. The original carboxylic acid groups that were on the palmitic acid molecules have reacted with the original alcohol groups that were on the glycerol molecule. Thus, a new functional group has been formed. This functional group is the *ester group*. This group reacts in a way that is different from either an alcohol or a carboxylic acid. All alcohols can react with carboxylic acids to form compounds called *esters*. *Glyceryl tripalmitate* is the palmitic acid triester of glycerol.

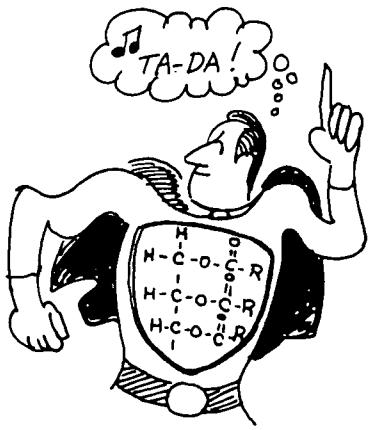
GLYCEROL $C_3H_5(OH)_3$



GLYCERYL TRIPALMITATE (a triglyceride) $C_{51}H_{98}O_6$



In your body, triglycerides serve as energy-storage compounds. When all carbohydrate stores are used up, the triglycerides come to the rescue. Enzymes first change the triglyceride molecule back to the component carboxylic acids and glycerol molecules. These are then converted by a long series of enzymatic reactions into water and carbon dioxide. Energy is given off in this process. Much of this energy is used to regenerate ATP, similar to the way in which the energy from glucose is used to produce ATP.

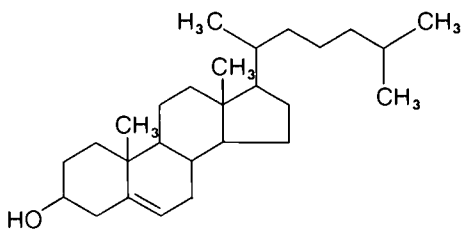


TRIGLYCERIDES TO THE RESCUE!

If you do not eat enough energy compounds, your body uses the glycogen stored in your tissues. When this is used up, your body draws from triglycerides stored in adipose tissue (fat) to keep the system going. This is why exercise can "melt" fat. Perhaps now you can understand why people who are on weight-reduction diets limit their intake of carbohydrates and fats.

Triglycerides and fatty acids are not the only compounds classified as lipids. Another example is *cholesterol*. This compound, like other lipids, is essentially all hydrocarbon. Because of this, cholesterol is always found with the other lipids extracted from natural sources. In fact, lipids are defined as naturally occurring compounds that can be extracted with "lipid solvents," such as hexane and chloroform. Lipid molecules can be recognized because they are mostly hydrocarbon and generally contain few functional groups. Because of this, they are generally insoluble in water.

CHOLESTEROL



Throughout our discussions we have repeatedly referred to the reactions that biomolecules undergo. Before carbohydrates or lipids can be used as sources of energy, a number of reactions must occur. We have mentioned that these reactions are catalyzed by enzymes. But what are these enzymes? In an earlier section we said that enzymes are actually proteins. Then what are proteins?

B-9 Proteins and Amino Acids

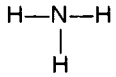
All of us have used the word *protein* at one time or another. But can we explain what the word means? The word is derived from the Greek *proteios*, which means "prime" or "chief." This name was chosen by early investigators who recognized that these compounds were of extreme importance in living things. In fact, protein is one of the chief biomolecules in all of the cells in our body. Compared with carbohydrates and lipids, proteins are exceedingly complex. In addition to the elements carbon and hydrogen, proteins contain oxygen and nitrogen. Many proteins also contain sulfur. Some proteins contain other elements such as phosphorus, iodine, or metal ions such as Fe^{+3} and Cu^{+2}.

These food groups—milk products (*right*) and meat, fish, poultry, eggs, and nuts—are important sources of protein, which contains nitrogen and amino acids essential for the growth and the repair of body tissue.

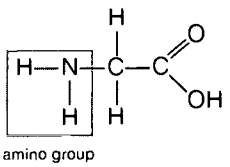
Although there is an extremely large number of different proteins, they all have one thing in common. All proteins are composed of *amino acids*. A protein is somewhat analogous to a long chain. The amino acids are the individual links in the chain. Since the amino acid is the fundamental unit in proteins, we must look closely at the amino acids before we can ask intelligent questions about proteins.

As you can see, the carboxylic acid group is the same one we have already seen in fatty acids. But what about the amino group? This is the first time we have seen the element nitrogen in a functional group. There is a great deal of similarity between the amino group and the inorganic compound called *ammonia*.

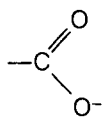
AMMONIA

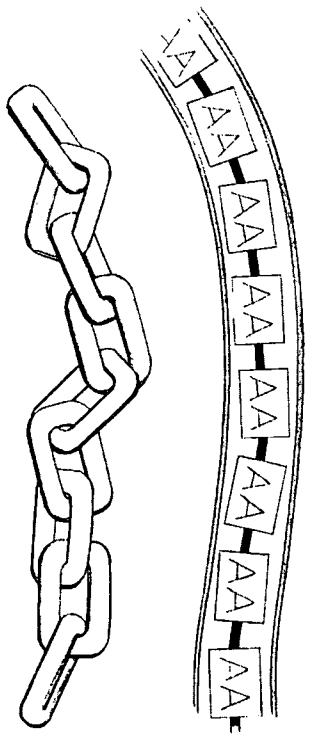


GLYCINE



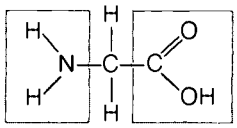
All amino acids contain at least two functional groups. We have seen one of these before. It is the group found in fatty acids:





Amino acids are the fundamental units in a protein. A protein has a chainlike structure. The amino acids can be looked upon as individual links in the chain.

GLYCINE

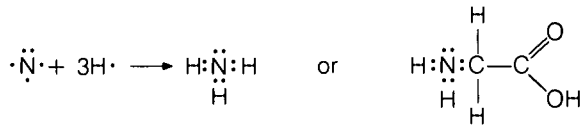


amino group

carboxylic acid group

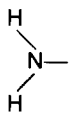
This explains why amino acids are called *acids*. However, amino acids are not classified with the fatty acids. This is because amino acids also contain the *amino* functional group. The simplest amino acid compound has been given the name *glycine*.

Nitrogen has five valence electrons, so it will form a stable compound when it shares three other valence electrons. In ammonia, the nitrogen is sharing three pairs of electrons with three hydrogen atoms. In the amino acid molecules, nitrogen also shares three pairs of electrons. One pair of electrons is shared with a carbon atom and two pairs of electrons are shared with two hydrogen atoms.

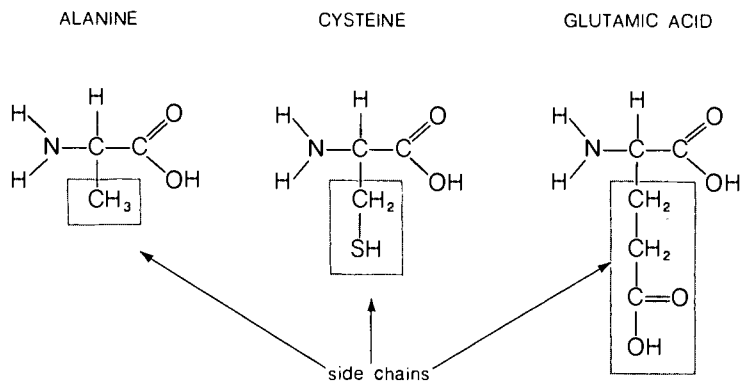


These electron-dot structures show all of the valence electrons around the nitrogen atom, including the unshared pair. In line structures, the unshared electrons are usually left out, but you must remember that these unshared electrons are present even though they are not shown.

amino group



Glycine is one of the twenty amino acids most commonly found in proteins. The structures of these amino acids are shown in Appendix II of this module. Three of these amino acids are shown in the following diagram. Compare their similarities and differences. Note that some amino acids contain functional groups in their side chains.

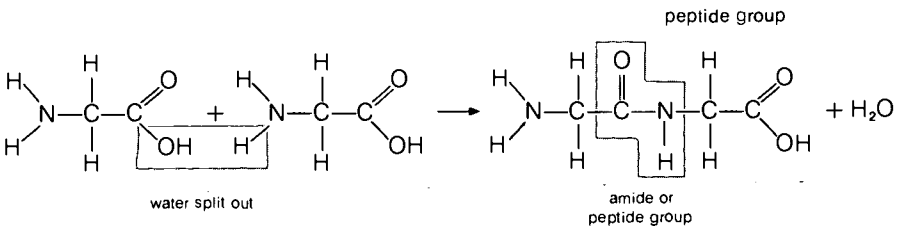


In one sense all of these compounds look much alike. They all have an amino acid and a carboxylic acid functional group attached to the same carbon atom. Nevertheless, each amino acid has a unique part that makes it different from all other amino acids.

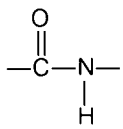
This unique part of the amino acid molecule is called the *side chain*. Later we will find that the different side chains are important in understanding properties of different proteins.

For the moment let us concentrate on the parts of the amino acid molecules that are identical, that is, the carboxylic acid and amino functional groups.

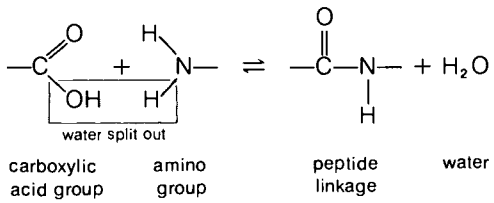
There is one extremely important reaction of amino acids that involves the carboxylic acid functional group in one molecule and the amino functional group in another. Let us illustrate this reaction between two molecules of the amino acid glycine.



In this reaction two molecules of glycine have reacted to produce a new compound. (In so doing, a molecule of water has been "split out.") In our starting materials, we had two amino functional groups and two carboxylic acid functional groups. In the product we have only one amino group and one carboxylic acid group. The other amino group and carboxylic acid group have been converted into a new functional group. This group is called the *amide group*, or the *peptide functional group*. The peptide group holds the two original amino acids together in the new compound. For this reason, this is often called the *peptide linkage*, or *peptide bond:*



In the process of forming this peptide linkage, water is split out of the old functional groups.*



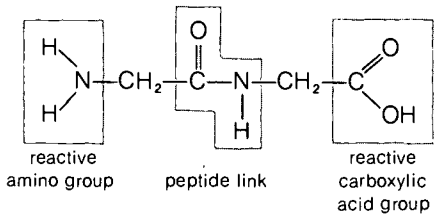
*As you might guess, water must be introduced again in order to break the peptide linkage. If this occurs, we regain the original functional groups. This process is called hydrolysis and we will have more to say about this later in the module.

The German chemist Emil Fischer was an early Nobel prize winner for his work in sugar and purine synthesis.

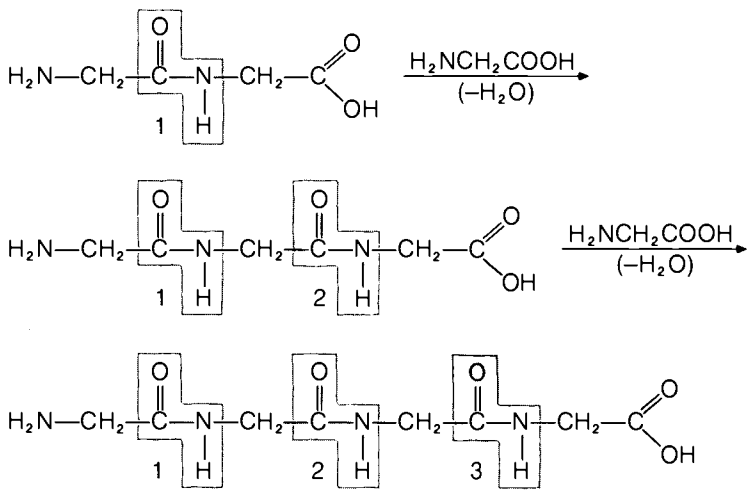
TIME MACHINE

1898	Teddy Roosevelt leads charge of Rough Riders up San Juan Hill in Cuba during the Spanish-American War.
1899	Jean Sibelius writes his first symphony.
1900	Ferdinand von Zeppelin launches first rigid dirigible airship.
1901	The first Nobel prizes are awarded.
1902	**Emil Fischer demonstrates peptide bond structure of proteins.**
1902	Alfred Binet devises "intelligence tests" and the "I.Q."
1903	Jack London writes *The Call of the Wild.*

Let us look a bit closer at the product formed when the two amino acids link together. Notice that the product still has a reactive amino group and a reactive carboxylic acid group.



Do you suppose that this molecule could react again with another amino acid to form a second peptide linkage? This is exactly what can happen. In fact, this type of reaction can occur over and over again until we have a very long string of amino acids linked together by peptide linkages.



1, 2, 3 = first, second, and third peptide linkages

If we continue linking amino acids together, we eventually form a protein. Thus, a protein is a long chain of amino acids linked together with peptide bonds. Most proteins contain hundreds of amino acids bonded together in this way. Because of their sheer size, we might expect the large macromolecules of protein to have some properties that are not shared by simple amino acids. But on the other hand, since amino and carboxylic acid groups are still present in the large protein molecule, proteins and amino acids might also have some properties in common. We'll pursue these ideas further in the following section.

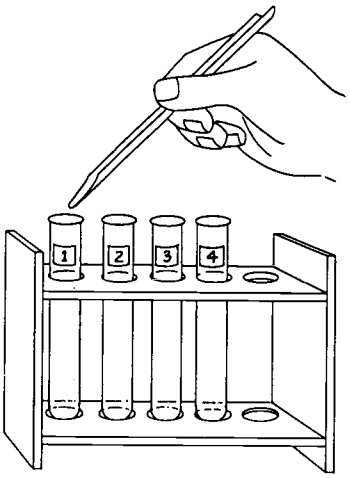
Properties and Reactions of Biomolecules

What do we know about the properties of biomolecules in general? We know that carbohydrates, proteins, and amino acids contain large proportions of functional groups. On the other hand, large parts of lipid molecules are hydrocarbon, and lipids have a small proportion of functional groups. Can this information help us to understand the properties of biomolecules? Keep this question in mind as you proceed.

By taking advantage of the properties and reactions of biomolecules, the analyst can better determine the clinical health of an individual.

EXPERIMENT

B-10 Solubilities of Biomolecules



In this experiment you will be concerned with the properties of some common biomolecules. Carefully record your observations. Then, using the information that you learned thus far, see if you can explain your observations.

You will test the solubility of four different biomolecules in different types of solvents. The biomolecules are

A. glucose, a monosaccharide
B. monosodium glutamate, an amino acid salt
C. gelatin, a protein
D. vegetable oil, a lipid

Set up a rack of four test tubes. Fill each test tube with 5 to 10 cubic centimeters (cm^3) of water (H_2O is a polar solvent*). Add a very small amount (the size of a match head or less) of sample A to the first test tube. Shake it well. Does sample A dissolve?

Repeat the test using sample B in the second test tube, sample C in the third test tube, and sample D in the fourth test tube.

Fill four different test tubes with 5–10 cm^3 of hexane (C_6H_{14} is a nonpolar solvent). Test each of the four samples, A, B, C, and D, for solubility in hexane.

Last of all, test to see if hexane will dissolve in water. Record your observations in a table. Which molecules are soluble in water? Which are soluble in hexane?

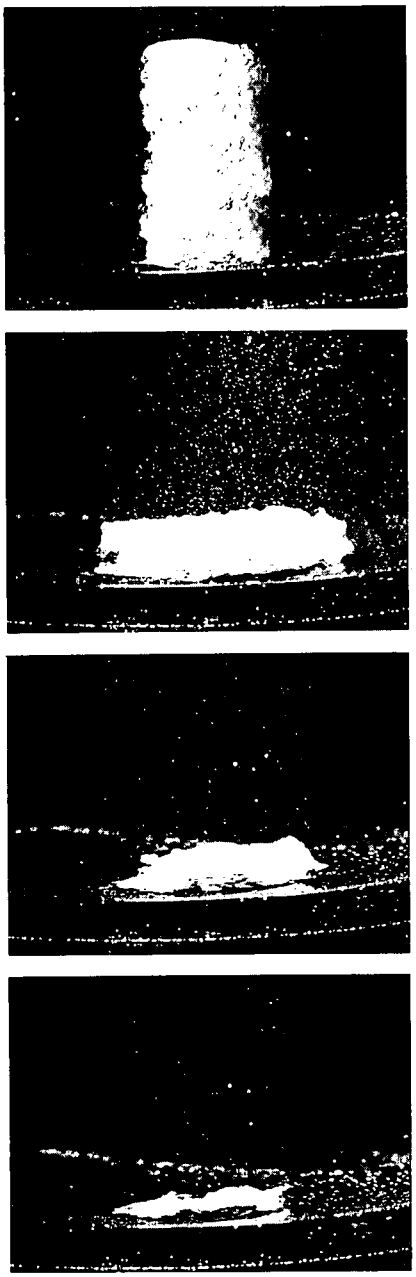
*Recall the meaning of the word *polar*. See *Reactions and Reason: An Introductory Chemistry Module*.

B-11 Like Dissolves Like

The conclusions drawn from many experiments similar to the one you have just finished conform to a very good "rule of thumb" for predicting solubilities of compounds: *Like dissolves like*. Can we explain how this rule works? Water can dissolve compounds that contain large numbers of functional groups. Our explanation is based on the knowledge that water is a polar solvent. Organic compounds that dissolve in water must be polar also. In the compounds that we tested in experiment *B-10 Solubilities of Biomolecules,* functional groups provided this polarity. If a large proportion of functional groups is present in a molecule, that molecule is generally soluble in polar solvents such as water.

On the other hand, organic molecules that lack functional groups are not polar. Solvents such as hexane are called *nonpolar solvents*. Most lipids, such as vegetable oil, are soluble in nonpolar solvents and insoluble in polar solvents. Vegetable oil is composed largely of triglycerides. These lipid molecules contain huge hydrocarbon tails. Because of the large proportion of hydrocarbon, triglycerides are relatively *nonpolar* substances.

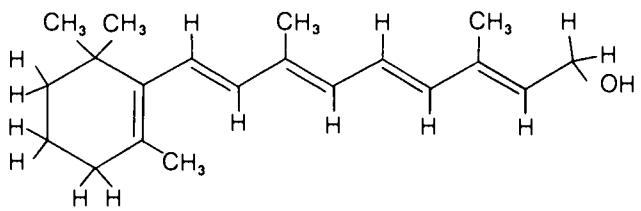
The phenomenon of solubility in water is extremely important in biological systems. Living things contain from 80 to 90 percent water. Thus, many biological reactions take place in water. Scientists find it useful to assign special names to characteristics of matter that they believe are important. For example, the class of compounds that are soluble in water is given the special name *hydrophilic*. This literally means they "love water." (Remember, like dissolves like.) In a similar sense, substances that are not soluble in water are known as *hydrophobic* compounds. Can you figure out the literal meaning of this name? Now we can say that hydrocarbons such as hexane and portions of the triglyceride molecule are hydrophobic. By contrast, amino acids and carbohydrates contain hydrophilic groups.



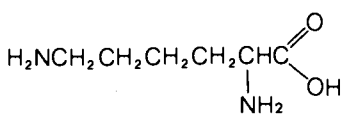
A sugar lump dissolving in water and breaking down into individual molecules. The sugar molecules are distributed among the water molecules.

EXERCISES

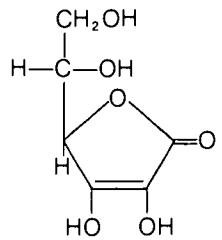
1. Define the terms "hydrophilic" and "hydrophobic" and give two examples of molecules showing these properties.
2. The structure of vitamin A is shown below. Predict the solubility of vitamin A in water and its solubility in hexane. Is this molecule hydrophobic or hydrophilic? To which class of biomolecules does this compound belong?



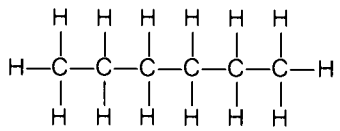
3. The compound shown below is called lysine. Locate and name the functional groups that are present. To which class of biomolecules does this compound belong?



4. The structure of vitamin C is shown below. Predict the solubility of this compound in water and hexane. To which class of biomolecules does this compound belong?



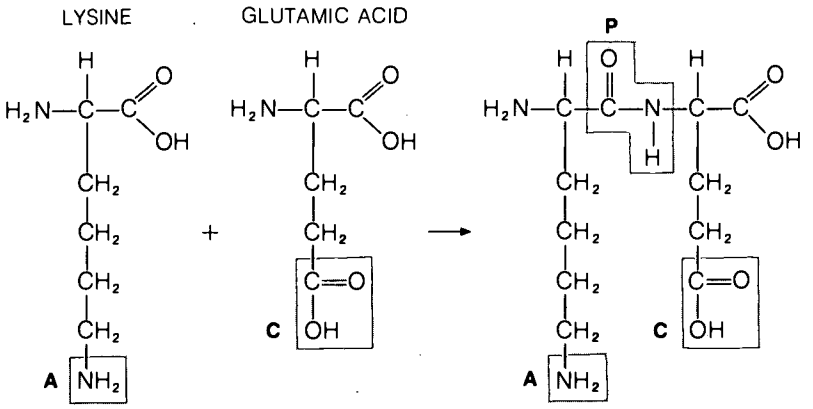
5. The molecular structure of hexane is shown below. Explain why it is a nonpolar solvent.



B-12 Identifying Biomolecules

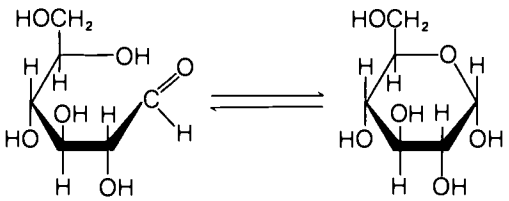
Simple physical tests such as solubility are often useful. By using this technique, we have tentatively established that vegetable oil contains lipids. However, the other classes of biomolecules are not readily identified by solubility alone. For example, both carbohydrates and proteins are soluble in water. The amino acid and the protein behaved similarly in experiment *B-10*. Clearly, other tests must be conducted before we can hope to identify biomolecules by type.

Let us recall what we know about some of these biomolecules. Glutamic acid, an amino acid, and gelatin, a protein, are closely related because of the functional groups that they contain. But both are quite different from carbohydrates. Will their chemical reactions be the same? The proteins have one special feature that the amino acids do not have: the peptide bond. However, proteins do have amino and carboxylic acid groups in some of the side chains. Thus, proteins will undergo many of the reactions that amino acids exhibit. But they will also participate in some special reactions of their own, resulting in part from the presence of the peptide linkage. The different reactions of amino acids and proteins can be used to tell them apart.



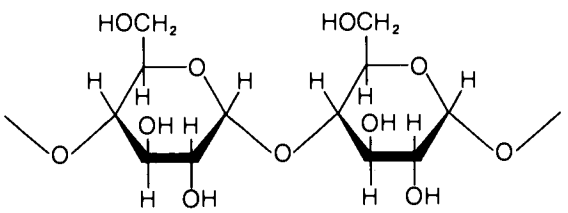
The relationship between glucose and starch is similar. Glucose is a sugar that has both alcohol groups (—OH) and an aldehyde group:

In this case, the chemistry is a little more complicated because glucose can exist in one of two structural forms, which are in *equilibrium* with each other when glucose is dissolved in water. This means that both forms of sugar are present in any glucose solution.



The second—or cyclic—form is combined in long chains to make starch. Although the first—or noncyclic—form of glucose has an aldehyde group, neither the cyclic form of glucose nor starch has an aldehyde group. Since starch will not undergo any reaction that depends on the presence of an aldehyde group, it can be distinguished from glucose. But both glucose and starch contain OH groups; so both molecules will participate in reactions that depend upon alcohol groups.

PORTION OF STARCH MOLECULE



Proteins and starch both have the ability to react with inorganic ions and molecules. The result is a new structure called a *complex*. These macromolecules are able to form complexes because they contain functional groups that can react with the inorganic ions. Macromolecules are flexible. Thus, they can fold up to bring the functional groups into the proper position to form complexes. Because proteins and starch have different structures, they form complexes with different ions and molecules. Some of these complexes are deeply colored. The specific reactions of proteins and starch to form different complexes with characteristic colors have been used as an analytical method for detecting and identifying macromolecules. To learn more about complexes, see *Diversity and Periodicity: An Inorganic Chemistry Module*.

Over the years, biochemists have developed a large number of chemical tests to study and distinguish biomolecules. In the next experiment you will conduct four of these tests on two small molecules and two macromolecules. All the tests depend on the chemical reactions of the biomolecules that we have discussed so far. The two small molecules are a sugar (glucose) and an amino acid salt (monosodium glutamate). The two macromolecules are a polysaccharide (starch) and a protein (gelatin).

EXPERIMENT

B-13 Chemical Reactions of Biomolecules

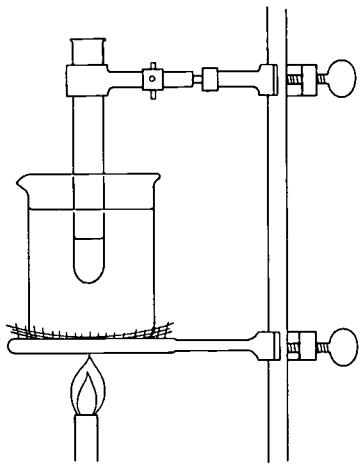
There are two parts to this experiment. In Part A you will work with known compounds (glucose, monosodium glutamate, starch, and gelatin) to determine their behavior in each of four tests. In Part B you will apply this information to test and identify an unknown sample.

Part A: Chemical Test Procedures

Working with a partner, conduct the iodine test, Benedict's test, the ninhydrin test, and the biuret test on each of the four compounds. (Each compound is already dissolved in water.) You will do a total of sixteen tests. Divide the work between you. Record your results in a table.

1. **Iodine test:** Using four clean test tubes, place 1 cm^3 of glucose solution in one test tube, 1 cm^3 of starch solution in another, 1 cm^3 of monosodium glutamate solution in a third, and 1 cm^3 of gelatin solution in a fourth test tube. Add a few drops of iodine test reagent (aqueous iodine) to each. Record the color.
2. **Benedict's test:** As in the iodine test, start with four clean test tubes and place 1 cm^3 of the samples in each. Add 1 cm^3 of Benedict's reagent [copper(II) citrate in sodium carbonate]. Heat for 5 minutes in a boiling water bath. Record any observations, including color changes.

3. **Ninhydrin test:** Starting with four more 1-cm³ samples, add 1 cm³ of ninhydrin solution and 1 cm³ of 10-percent pyridine to each sample. Heat in a boiling water bath for 5 minutes. Record your observations.
4. **Biuret test:** In each test tube, mix 1 cm³ of sample and 1 cm³ of biuret reagent [alkaline copper(II) tartrate]. Record your observations.



Part B: Identifying Unknowns

Since each compound in Part A produced a color with only one or two tests, you can use these tests to identify these specific compounds. Identify your unknown sample using the four tests (with four clean test tubes). The unknown will be one of the four biomolecules that you tested in Part A. What was your unknown? What evidence do you have?

Construct a table similar to the following to record the results of your tests.

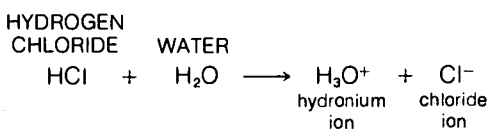
	TESTS			
Sample	Iodine	Benedict's	Ninhydrin	Biuret
Monosodium glutamate				
Gelatin				
Glucose				
Starch				
Unknown				

Questions:

1. Egg white is primarily protein and water. Predict its behavior using each of the four tests in experiment *B-13.*
2. In the iodine test can the reaction observed be attributed to the alcohol groups? Explain.

B-14 Amino Acids: Basic and Acidic Facts

We have already discussed one important reaction of amino acids—the formation of the peptide bond. Amino acids also undergo another important type of reaction called an acid-base reaction. Do you remember what an acid is? Hydrogen chloride is an example of an acid.



TIME MACHINE

1921	Introduction of injection-molding technique makes possible large-scale production of plastic products.
1922	The tomb of Tutankhamun ("King Tut") is discovered in Egypt by Lord Carnarvon and Howard Carter.
1923	**Johannes Brønsted and Thomas M. Lowry independently define acids as proton donors and bases as proton acceptors.**
1924	Sigmund Romberg's operetta, *The Student Prince*, opens in New York.
1926	Hirohito becomes emperor of Japan.
1927	Charles Lindbergh makes first transatlantic solo flight.

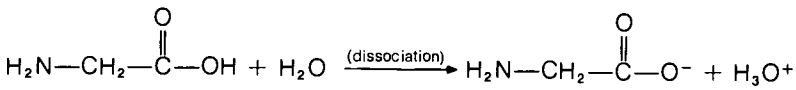
The preceding reaction shows that hydrogen chloride gas breaks apart when it is dissolved in water. We say that hydrogen chloride dissociates into a hydrogen ion (H^+) and a chloride ion (Cl^-).

$$HCl \xrightarrow{\text{(dissociation)}} H^+ + Cl^-$$

When the reaction occurs in water, the hydrogen ion (H^+) is transferred from hydrogen chloride to water to form the hydronium ion (H_3O^+).

$$H^+ + H_2O \longrightarrow H_3O^+$$

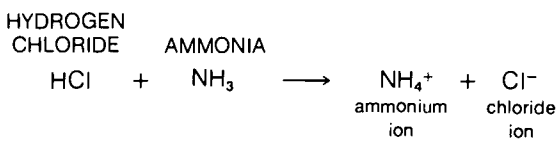
How does this relate to amino acids? Let's look at the compound glycine. We know that it has a carboxylic acid group. What do you suppose happens to this group when it is dissolved in water?



The carboxylic acid group dissociates. Just as we noted with HCl, the hydrogen ion produced is transferred to a water molecule. Both HCl and glycine are acids because they donate hydrogen ions to another substance. In fact, this is one definition of an acid.

An acid is any substance that will donate a hydrogen ion (H^+) to another substance.

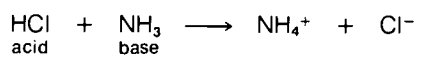
In both of the preceding cases, the substance that accepted the hydrogen ions was water. But other substances can accept hydrogen ions also. For example:



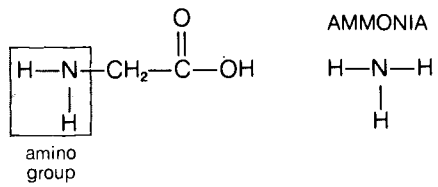
We have said that the substance that donates a hydrogen ion is called an acid. But what do we call the substance that *accepts* a hydrogen ion?

Any substance that will accept a hydrogen ion (H^+) from an acid is called a base.

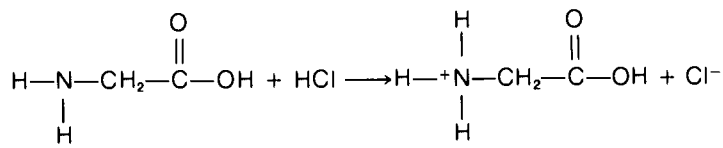
Ammonia is a base because it will accept a hydrogen ion from the acid hydrogen chloride.



Recall that the amino group in amino acids is very similar to the ammonia molecule.



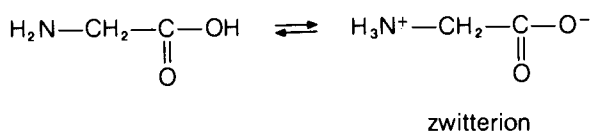
If ammonia is a base, what about the amino group in amino acids? It should also be a basic group and that is exactly what it is. An amino group can accept a hydrogen ion from an acid just as any other base can.



B-15 Zwitterions: Negative and Positive

You have seen that an amino acid contains both an acidic group and a basic group. This means that an amino acid can function as a base in a reaction with an acidic compound, or it can function as an acid in a reaction with a basic compound.

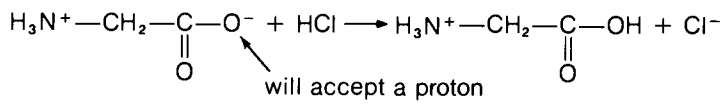
This leads to an obvious question. If the same molecule has both acidic and basic groups, why doesn't it react with itself? Well, it does. The product of such a reaction is called a *zwitterion*. In fact, in most biological systems, proteins and amino acids exist as zwitterions.



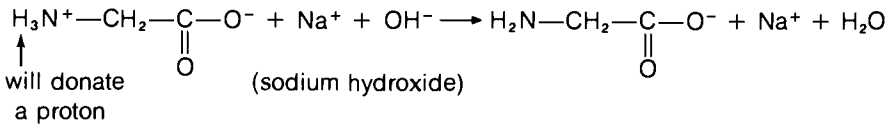
A zwitterion is a molecule that has both positive and negative charges. The presence of charged groups clearly suggests that a zwitterion such as glycine should behave in some ways like an *ionic* substance. This is an important observation. It helps to explain certain properties of amino acids. For example, the melting points of the amino acids are much higher than you would predict from their small sizes. Small covalent compounds usually have low melting points. In fact, a molecule the size of glycine should

be a liquid at room temperature, just as acetic acid (CH_3COOH) is a liquid at room temperature. However, glycine is a solid with a melting point between 232–236°C. The fact that glycine is a zwitterion provides an explanation. As a zwitterion, glycine behaves like an ionic compound. Ionic compounds have high melting points. For example, sodium chloride (NaCl) has a melting point of 801°C.

It is important to realize that the zwitterion still has acidic and basic groups. But the acidic and basic roles are now associated with different groups. Whereas the amino group was basic in the original compound, *the carboxylate group is basic in the zwitterion.*

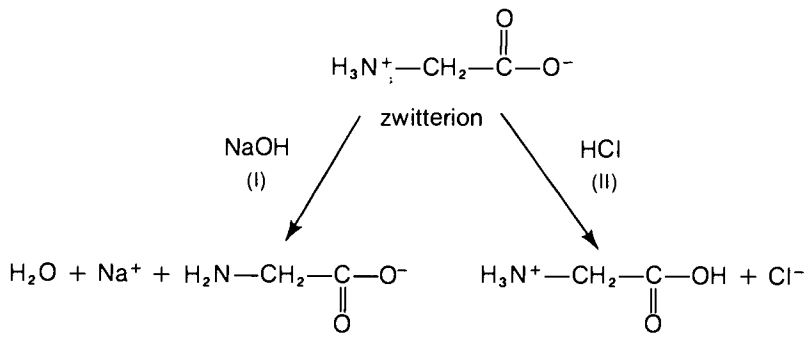


Similarly, while the carboxyl group in glycine was acidic, *the ammonium group is acidic in the zwitterion.*



Notice that the reactions match our definitions of acids and bases. The carboxylate ion acts as a base because it accepts a hydrogen ion from hydrogen chloride. The ammonium group acts as an acid because it donates a proton to the base OH^- from sodium hydroxide.

Proteins and amino acids exist as zwitterions in biological systems. Therefore, the important acid-base reactions are the reactions of the zwitterion. In these reactions, the ammonium group is an acid and the carboxylate group is a base.



If the zwitterion is reacted with a base such as sodium hydroxide, we observe reaction I. When the zwitterion is treated with acid, such as hydrochloric acid, we observe reaction II. These are the same kinds of acid-base reactions that the zwitterionic forms of proteins also undergo. As we shall see in the next section, the acid-base reactions of proteins are of extreme importance in understanding the function of enzymes.

EXERCISES

1. For each of the following reactions, identify which compound or ion is acting like an acid and which is acting like a base.

EXAMPLE:

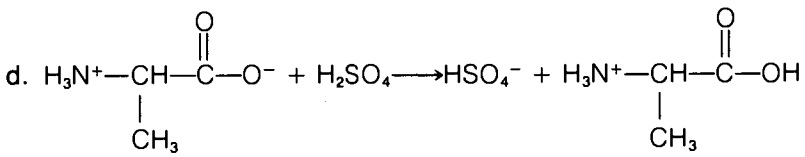
$HCl + NaOH \rightarrow NaCl + H_2O$ (HCl is the acid because it donates an H^+ and NaOH is the base because it accepts the H^+.)

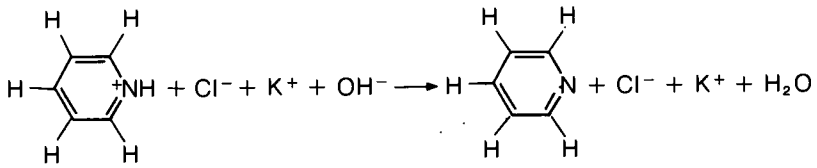
NOW TRY THESE:

a. $CH_3NH_2 + HNO_3 \longrightarrow CH_3NH_3^+ + NO_3^-$

b. $HCl + H_2O \longrightarrow H_3O^+ + Cl^-$

c. $S^{2-} + H_2O \longrightarrow OH^- + HS^-$

d. 

e. 

f. $H_3N^+CH_2COOH + 2CH_3—NH—CH_3 \longrightarrow H_2N^-CH_2COO^- + 2CH_3—N^+H_2—CH_3$

2. Look at the compounds in exercise 1. Identify any which are zwitterions.

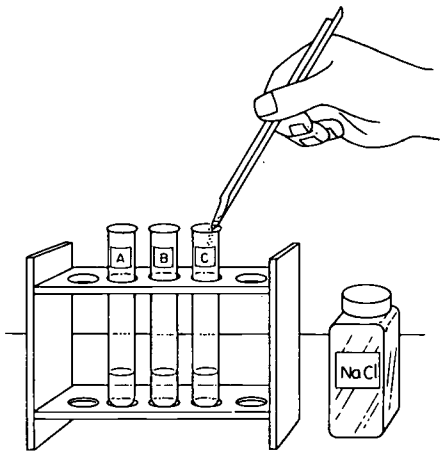
Enzymes: Where the Action Is

Enzymes are one of the most extensively studied classes of biomolecules. Literally thousands of enzymes have been discovered and investigated. But what are enzymes? What do they do? How do they work? Chemically speaking, enzymes serve as catalysts—and they are by far the most important catalysts in biochemistry.

A scientist fits a "molecule" into an enzyme model that he is constructing. Building and studying models are important steps in providing information about the structures of biomolecules.

B-16 Catalysts and Reaction Rates

miniexperiment



All catalysts, including enzymes, have two important properties: (1) they increase the rate of a reaction, and (2) they are not used up in the reaction.

In this activity, you will examine the effects of some materials on a reaction and determine which materials can catalyze the reaction. The reaction you will observe is the breakdown of hydrogen peroxide to form water and oxygen.

$$H_2O_2 \longrightarrow H_2O + O_2$$ (Can you balance this equation?)

You can observe the progress of this reaction by watching the bubbles of oxygen that form in the solution. Without a catalyst, this reaction is so slow that you may not see any bubbles forming.

Put about 1 cm^3 of 3-percent hydrogen peroxide solution in each of three clean test tubes. Label them A, B, and C. Add a few grains of manganese dioxide (MnO_2) to test tube A, a drop of fresh liver juice to test tube B, and a few grains of sodium chloride to test tube C. Record your observations and determine which of these materials acts as a catalyst in this reaction. Explain what the catalyst does to the reaction.

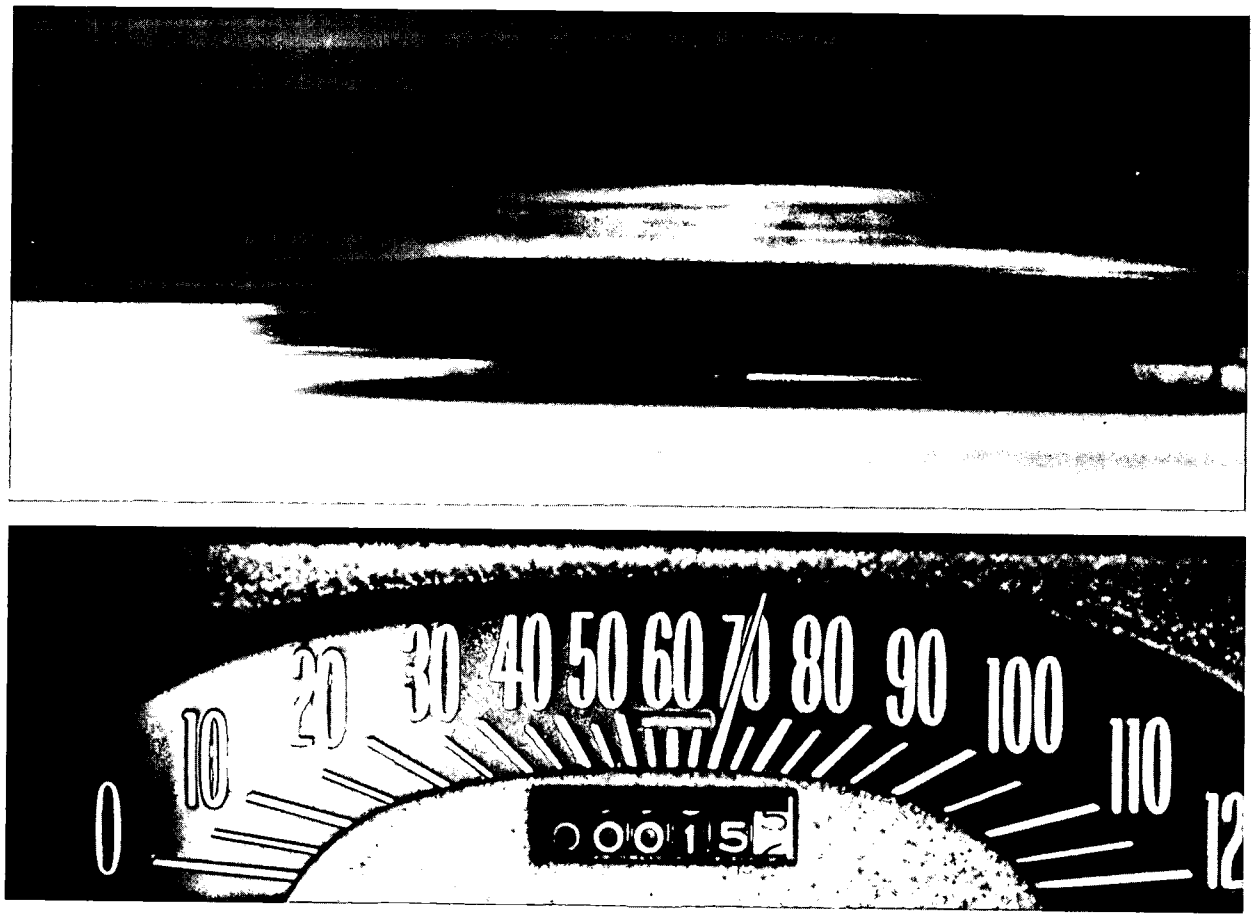
Next place a few grains of manganese dioxide in a clean test tube and 2 drops of fresh liver juice in a second test tube. Heat both test tubes in a boiling water bath for 3 minutes, remove them, and allow them to cool to room temperature.

After the test tubes have cooled, add about 1 cm^3 of 3-percent hydrogen peroxide to each. Record your observations. From your results determine one way that an inorganic catalyst and an enzyme are different. (This difference is one of the things we will want to explain.)

B-17 Characteristics of Catalysts

The factor in the liver juice that catalyzed the reaction is an enzyme called *catalase*. It is found in many organisms, including human beings. Have you ever cut yourself and used hydrogen peroxide to clean the wound? If you have, perhaps you have seen bubbles of oxygen coming from the wound. They were produced by the same enzyme reaction that you observed in the experiment.

The miniexperiment illustrates the first important property of catalysts. They increase the *reaction rate*. The reaction rate is commonly expressed as the amount of product produced in a



The speed of a car (kilometer/hour) is analogous to the rate of a reaction, which is often given in mole/minute. Something is changing per unit of time in both cases.

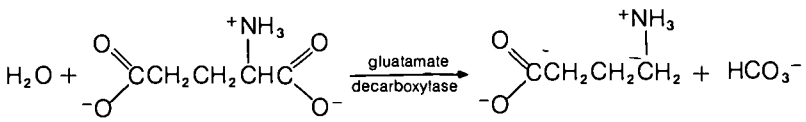
given period of time. For example, the reaction rate in the hydrogen peroxide experiment could have been defined as the number of moles of oxygen produced per minute. The reaction rate is somewhat like the speed of a car. You state the speed of a car in kilometers per hour and the reaction rate in moles per minute. The more moles of oxygen produced in a minute, the greater the reaction rate.

Catalase clearly caused a great increase in the reaction rate. This is typical of enzymes. In the absence of an enzyme, certain reactions would not be completed within days or even years. But with only a few micrograms of an enzyme such reactions might end within minutes. (You would need a magnifying glass to see a microgram of enzyme.)

Enzymes also exhibit the second property of catalysts: they are not consumed by the reaction. An enzyme reacts *temporarily* with a molecule of the starting material and helps convert it to the product. The enzyme itself is left unchanged by this process.

Thus, it is able to react with molecules of the starting material over and over again. A single enzyme molecule can react with millions of molecules of starting material (one at a time) without being changed.

Thus, enzymes have both properties common to all catalysts. They change the rate of the reaction without being used up in the reaction. But enzymes have another property that many other catalysts do not have. Enzymes have a high degree of specificity for certain reactions. This means that an enzyme will catalyze only one reaction (or possibly a small number of closely related reactions). For example, the enzyme *glutamate decarboxylase* removes one of the carboxyl groups from the amino acid—glutamate.*



water glutamate ⟶ aminobutyrate + bicarbonate

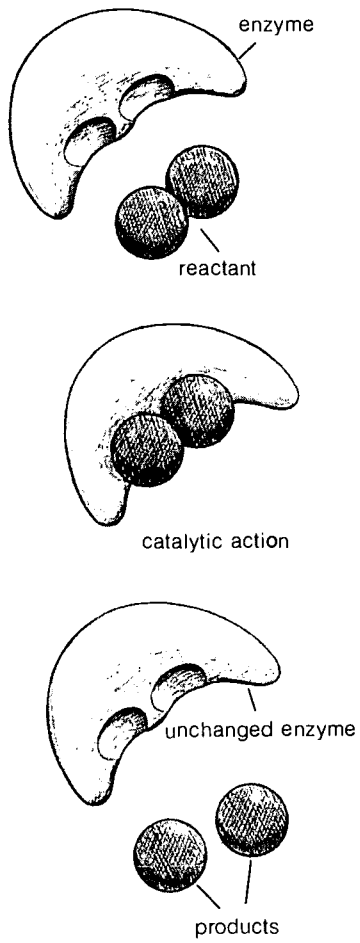
However, the enzyme is *so specific* for this reaction that it will not remove the carboxyl group from the amino acid aspartate. As you can see in the figures in the margin, the structures of aspartate and glutamate are almost identical. The only difference is that glutamate has one more CH_2 group than aspartate.

Because of their high degree of specificity, enzymes are usually named for the reaction they catalyze. The ending *ase* is added to indicate that it is an enzyme. You can tell from its name that glutamate decarboxylase is an enzyme that decarboxylates glutamate. However, some enzymes that were discovered and named in the early days of biochemistry are still known by their original names. Catalase is one of these. It is not named for the reaction it catalyzes. Other examples of enzymes that are not named for the reactions they catalyze are the digestive enzymes *pepsin*, *trypsin*, and *ptyalin*. You cannot tell from their names that they are enzymes. Their names do not even end in *ase*.

The catalytic properties of enzymes are important in cells. Since enzymes greatly increase reaction rates without being used up in reactions, cells need to produce only small amounts of any particular enzyme. The enzyme molecules that are produced may last in the cell for days or weeks. Cells must produce thousands of different enzymes, however, because each enzyme is very specific and catalyzes only one reaction.

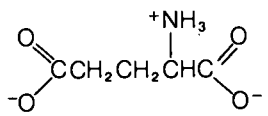
*We use the name glutamate rather than glutamic acid to indicate that the molecule has a net negative charge in biological systems. The name implies that a positive ion (such as Na^+ or K^+) is also present, but to simplify the discussion we have not included it. This terminology will be used throughout the module.

ENZYME REACTION

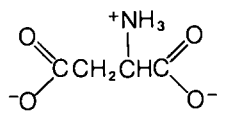


The general sequence of events during an enzyme-catalyzed reaction. First, the reactant is bound to the enzyme, then it undergoes the reaction, and, finally, the products are released. The enzyme is unchanged by the process.

GLUTAMATE



ASPARTATE

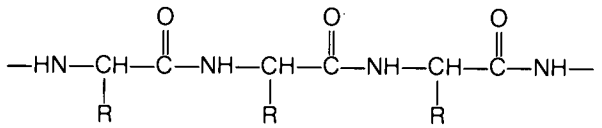


A scientist contemplates the abstract beauty in a model of one of the complex molecules of life—the enzyme, lactate dehydrogenase. The model is projected onto an electron-density map. This map shows the positions of the atoms in the enzyme, thus providing important information for the understanding of this biomolecule. (Another view of this same model is on page 38.)

B-18 Molecular Architecture of Enzymes

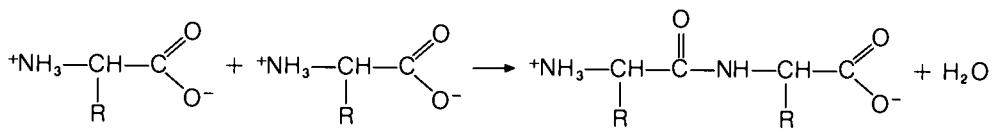
So far we have learned what an enzyme does. We know that enzymes are catalysts and that they are very specific. These are facts. But how do we explain these facts? Biochemists have found explanations by studying the structures of enzymes.

Many enzymes have been isolated and purified. All of these enzymes have been found to consist chiefly of protein. We can say that the primary structure of an enzyme is a long chain of amino acids held together by peptide (amide) linkages.



R = side chain of each amino acid

As we pointed out in an earlier section, the peptide linkage is formed by the reaction of the amino group of one amino acid with the carboxylic acid group of a second amino acid. A molecule of water is lost when the two groups combine.



If two enzymes catalyze different reactions, they must have different structures. But how can the structures of two enzymes be different? In fact, they can be different in several ways. One way is that different enzymes can have different numbers of amino acids. For example, catalase contains about 2160 amino acids per molecule. But the enzyme *ribonuclease* contains only 124 amino acids per molecule. (Ribonuclease is the enzyme that breaks down RNA.) Catalase contains about twenty times the number of amino acids that ribonuclease has. It is obviously a much larger molecule.

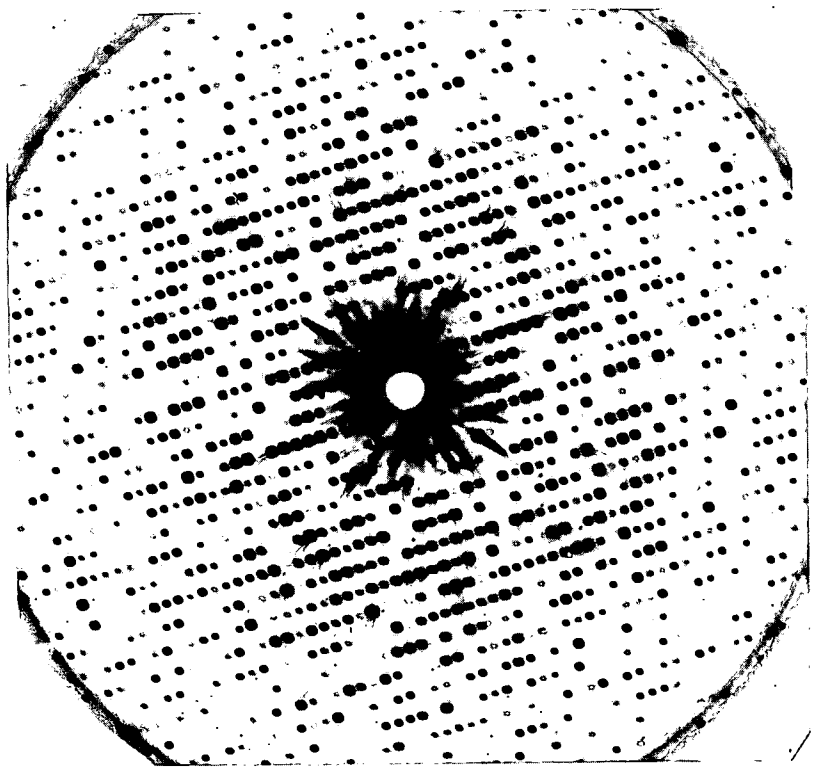
Knowing the number of amino acids in an enzyme does not tell us very much about the shape or strùcture of the enzyme. All that it tells us is whether the enzyme is large or small.

Actually we know much more about the structure of many enzymes. In many cases, we know the exact *sequence* of amino acids in the protein chain. This means that we know which amino acid is in each position in the chain. We can start at one end of the molecule and name each amino acid as we come to it.

Every enzyme has a unique sequence of amino acids. The sequence of amino acids in ribonuclease is different from the sequence in any other enzyme. Even if different enzymes were to contain the same number of amino acids, the amino acids in each enzyme would be put together in a different sequence.

TIME MACHINE

1923	Felix Salten publishes *Bambi.*
1923	Lee De Forest shows the first sound-on-film movie.
1925	F. Scott Fitzgerald publishes *The Great Gatsby.*
1926	**J. B. Sumner prepares the first crystals of an enzyme, urease, and proves it to be a protein.**
1926	Robert H. Goddard demonstrates the first liquid-fueled rocket.
1927	Babe Ruth hits 60 home runs in one season.
1928	Alexander Fleming discovers penicillin.

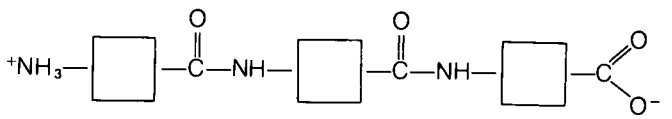


An X-ray diffraction pattern obtained from a myoglobin crystal has helped biochemists to learn about the structure of myoglobin. Scientists measured the position and intensity of each spot. This information was then used in complex computer calculations to reveal the positions of the individual atoms.

You might wonder if it is possible to combine amino acids in enough different sequences to make thousands of different enzymes. The answer is yes, and you can prove it to yourself by the following paper and pencil experiment.

Suppose each block in the figure below is a position in a protein chain. It is only three amino acids long—a very short protein molecule, indeed. Suppose each position in the chain could be filled with either amino acid A or amino acid B.

1. How many possible sequences are there? You can use A or B as many times as you like. For example, one sequence is BBA; another is ABB. There are others.



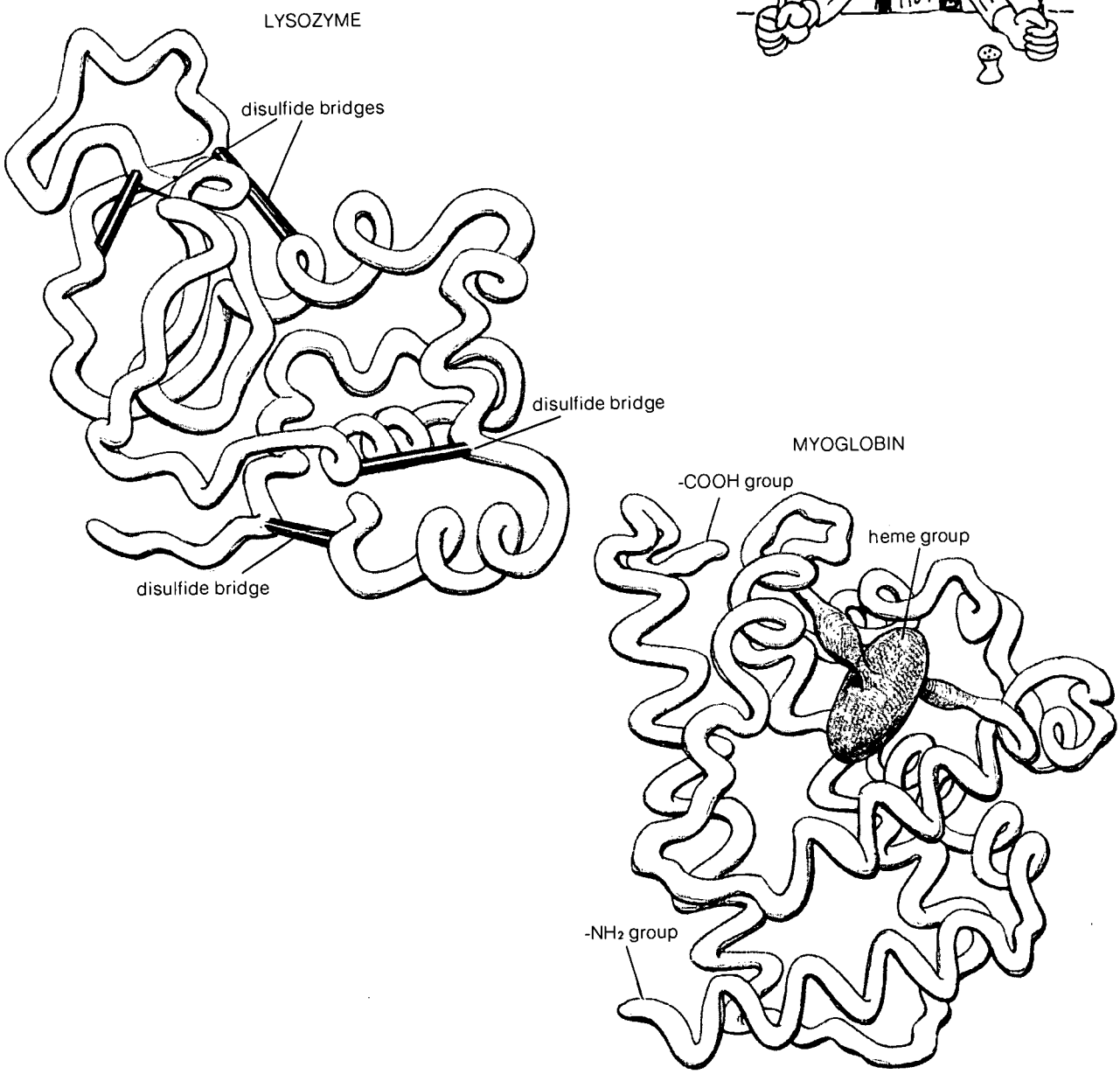
2. How many possible sequences are there if each position could be filled with one of three amino acids (A, B, or C)? For example, one sequence is CCC; another is ABC.
3. Now suppose that each position could be filled with one of the twenty common amino acids. How many possible sequences would there be?

No matter what your answer was to question three, you can see that the number of possible sequences is extremely large even though the protein is only three amino acids long. The number of possible sequences in a protein one hundred amino acids long is 20^{100}. That is an absolutely astronomical number. We will never have to worry about running out of sequences. You can see why proteins can do so many different jobs. There are almost an infinite number of them.

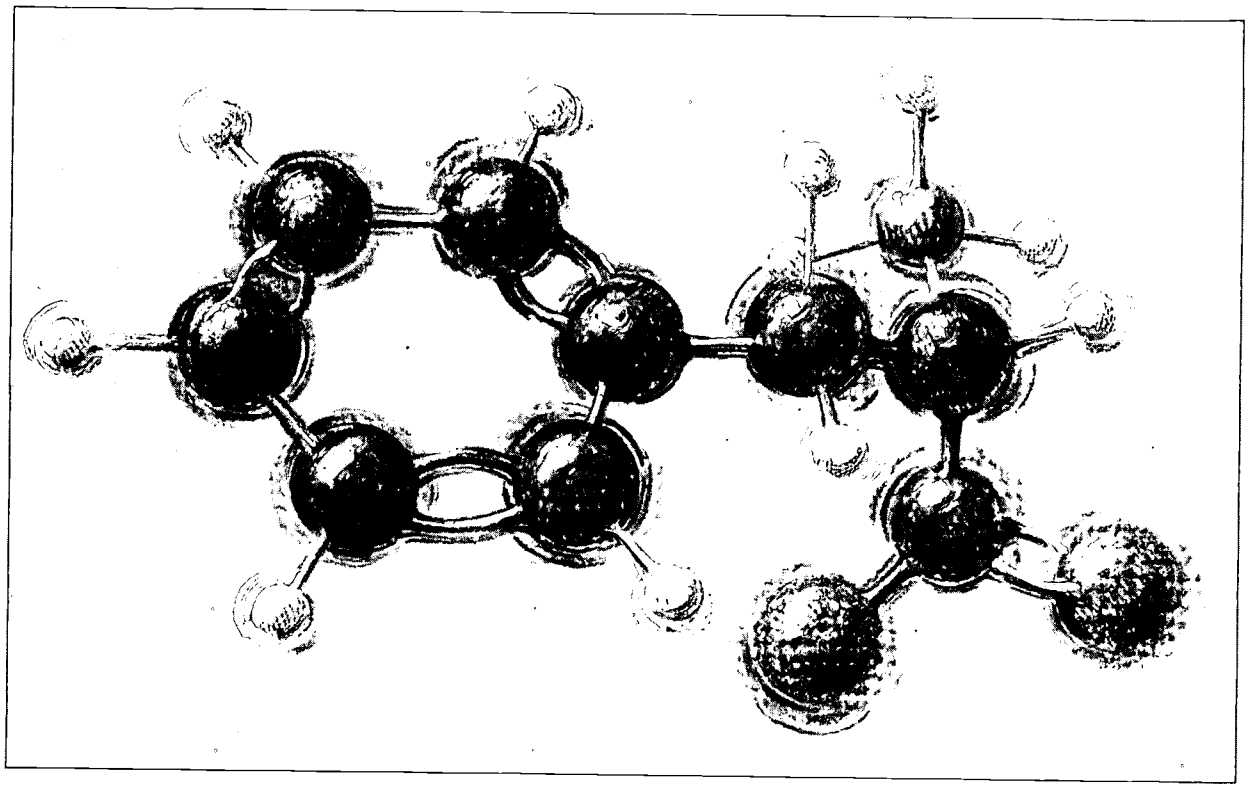
B-19 Holding the Folding

There is more to protein structure than the sequence of amino acids in the chain. Chains of amino acids are long and flexible. They are like string: they can fold and twist many ways. However, the amino acid chain of every protein is folded in a specific way. Since it takes years of work to determine the folded structure of an enzyme, the exact way the chain is folded is known for only a few of the thousands of enzymes.

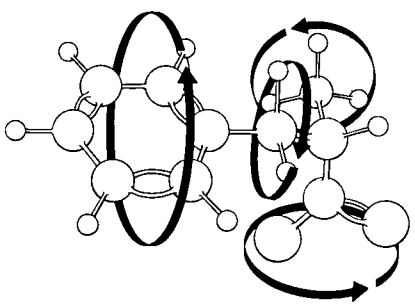
The folding of the enzyme *lysozyme* and the protein *myoglobin* are shown in the following illustration. You can see that lysozyme and myoglobin have very different structures. They also have different biochemical functions. Myoglobin is similar to hemoglobin. It helps to carry oxygen in the muscles and is the material that gives muscles their red color. Lysozyme is an enzyme that catalyzes the breakdown of the cell walls of many kinds of bacteria. It is found in many places, including tears and egg whites. Lysozyme acts like an antibiotic by destroying the cell walls of bacteria that may get into fluids such as tears and egg whites.



Let's return to the structures of these proteins and focus our attention on the individual atoms. We usually talk about the atoms within molecules as though they were motionless. This is not true. The atoms in molecules are continuously in motion. They can vibrate and rotate. The following figure of phenylalanine illustrates the kind of motion we are talking about.



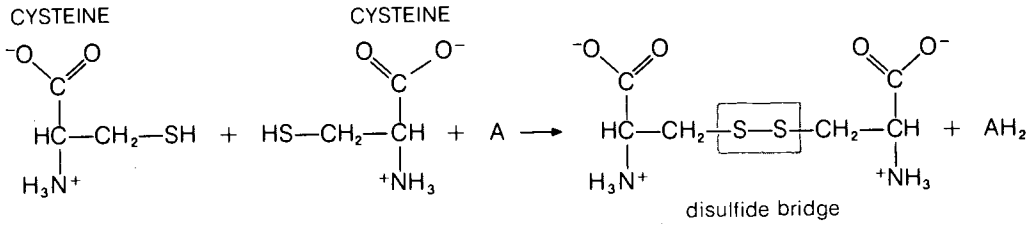
Atoms in molecules are always vibrating, as shown in the figure above. Groups connected by single bonds can rotate around the bond, as shown in the figure below.



If there is all this motion in a small molecule such as phenylalanine, imagine the amount of motion that is possible in a molecule, such as a protein, that is hundreds of times larger. You might expect that the whole protein chain could vibrate and rotate, giving the protein the appearance of a snake, wiggling and twisting. But we know that this is not true because the amino acid chains of proteins are folded in definite ways. We know that every myoglobin molecule is folded in exactly the same way. Thus there must be something that prevents a great deal of the motion of the chain and that holds it in its proper folded position.

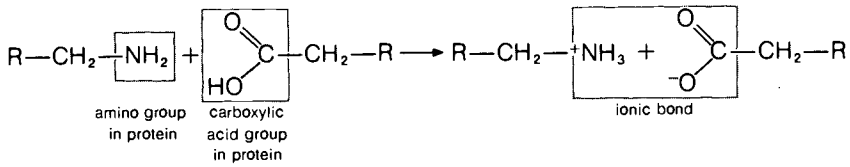
What keeps a protein in its proper shape? Several bonds and forces are involved. These are (1) the disulfide bridge, (2) the ionic bond, (3) the hydrogen bond, and (4) the hydrophobic bond.

The *disulfide bridge* is formed by a reaction between the side chains of *cysteine* molecules in different parts of the protein chain. The —SH group on the side chain of cysteine is called a *thiol group*. The two thiol groups can react to form a *disulfide bridge*. The reaction involves a third molecule which accepts the hydrogen atoms that are lost when the disulfide bridge is formed. Many molecules can do this. We have simply written the third molecule as A (for hydrogen *A*cceptor) to keep the reaction simple.



This reaction is common in proteins, and many proteins contain several disulfide bridges. If you look back at the diagram illustrating the structure of lysozyme, you can see that it has four disulfide bridges. The bridges connect distant parts of the molecule and form loops in the structure of lysozyme. The bridges are clearly holding the chain in a folded structure.

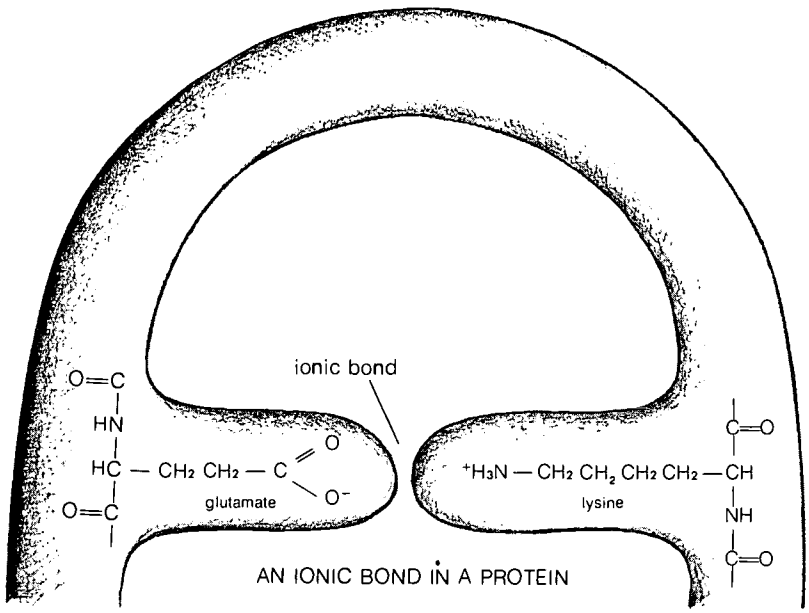
The second type of bond which helps to maintain the folding of the protein chain is the *ionic bond*. As you know, the amino group is a base and will take a hydrogen ion from an acid. This gives the amino group a positive charge.



On the other hand, the carboxylic acid groups are acids and will give up hydrogen ions. When they do, they are left with a negative charge. The positively charged amino group and the negatively charged carboxyl group are attracted to each other. This attraction results from the electrical attraction between opposite charges. It is similar to the attraction between sodium ions and chloride ions in sodium chloride crystals. The name *ionic bond* reminds us that the bond results from the attraction of oppositely charged ions.

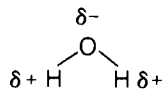
The ionic bonds in proteins are between amino acids that contain acidic and basic groups in their side chains. The acidic group in one amino acid forms an ionic bond with a basic group from another amino acid. The following figure illustrates the formation of an ionic bond between the amino acids lysine and

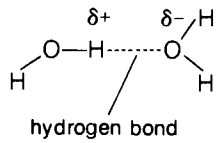
glutamate. The two amino acids forming the bond are separated by a long piece of the amino acid chain. Because of this, the chain must fold for the ionic bond to form. Once the chain folds, the ionic bond helps to keep it folded.



The third kind of bond that helps to keep the chain folded is called the *hydrogen bond*. This name is a little misleading. The term *hydrogen bond* does *not* mean the ordinary covalent bond between hydrogen and another atom such as carbon, oxygen, or nitrogen. Instead, a hydrogen bond is a much weaker bond.

In order to understand the hydrogen bond, we need to study the covalent bond in a little more detail. Covalent bonds result from the sharing of electrons between atoms. But the electrons are not shared equally by both atoms. Some elements tend to hold electrons more tightly than others. Oxygen is an example. The shared electrons in the covalent bond between oxygen and hydrogen spend more time closer to the oxygen atom. Since electrons are negatively charged, the oxygen in a covalent O—H bond will actually carry a slight negative charge. On the other hand the hydrogen atom attached to the oxygen will carry a slight positive charge.





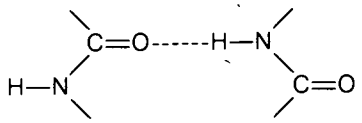
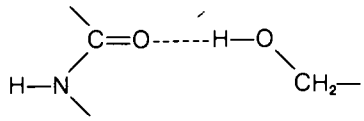
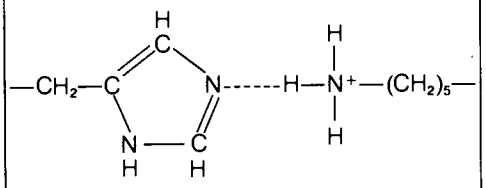
The hydrogen and the oxygen atoms in water carry slight electrical charges, as shown by the symbols δ^+ and δ^-. The electrical attraction between oppositely charged atoms on different molecules is called the hydrogen bond.

But how does all this help to explain the hydrogen bond? In the case of water, each hydrogen bond is formed between *two* molecules. The weak positive charge on the hydrogen in one molecule is attracted to the weak negative charge on the oxygen in another molecule. The attraction between the molecules results from the attraction that opposite electrical charges have for each other. Thus, the hydrogen bond is similar to the ionic bond between sodium ions and chloride ions. However, the hydrogen

bond is much weaker because the electrical charges on hydrogen and oxygen in compounds are much smaller than the charges on sodium and chloride ions.

The hydroxyl group is not the only functional group that can form hydrogen bonds. Some other examples of hydrogen bonds that can form in proteins are shown in Table 2. All of these hydrogen bonds are formed between atoms that carry small electrical charges. You can see from the table that many functional groups in proteins can form hydrogen bonds.

TABLE 2: SOME HYDROGEN BONDS IN PROTEINS

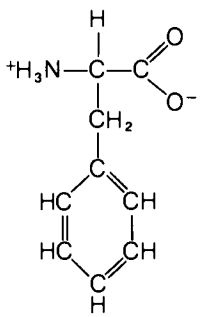
Structure	Hydrogen Bonds Between
	two peptide linkages
	hydroxyl group and peptide linkage
	histidine and lysine side chains

As you might guess, proteins contain large numbers of hydrogen bonds because so many groups can form them. Proteins also contain many ionic bonds. However, it is impossible to say exactly how many hydrogen bonds or ionic bonds are present in most proteins because we still do not know the position of each atom in the folded structure.

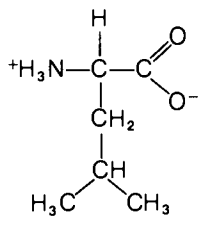
So far we have seen that disulfide bridges, ionic bonds, and hydrogen bonds are important in keeping a protein folded. But there is a fourth factor involved also.

What is this fourth factor? We call it the *hydrophobic bond.* The hydrophobic bond involves parts of the molecule that do not contain any functional groups. Several amino acids contain hydrocarbon side chains. For example, the side chains of phenylalanine and leucine are hydrocarbons. As you know, hydrocarbons are not very soluble in water and are said to be hydrophobic. The same can be said of these side chains. (Remember, like dissolves like.)

PHENYLALANINE



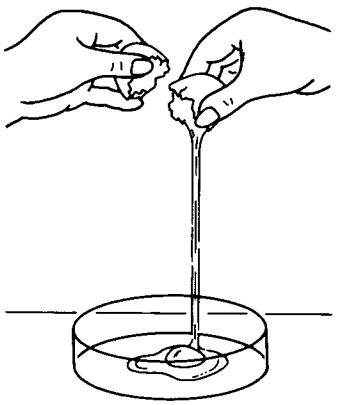
LEUCINE



You will recall from an earlier experiment that hexane and triglycerides did not dissolve in water. Instead, these nonpolar molecules stayed together in a layer. In a similar way, the hydrocarbon side chains of the amino acids tend to stay together inside the folds of the protein. In this way, they have the least contact with water. In a sense this forces the protein to fold in a specific way so that the hydrophobic side chains are close together. This tendency of the hydrophobic portion of the molecule to come together inside the folds is called the *hydrophobic bond.*

miniexperiment

B-20 Sunnyside Up or Poached



Egg white is a concentrated protein solution. Have you ever thought about what happens to an egg when you cook it? Of course, it solidifies. But why? Can anything else cause similar changes? Consider these questions during this experiment.

Add about 75 cm^3 of water to a 150-cm^3 beaker and heat it to at least 80°C. Obtain some egg white by breaking a fresh egg into a shallow container. Carefully separate the egg white from the yolk and place the egg white in a small beaker. You may wish to use an eye dropper for this purpose. Discard the yolk. (*Note:* One egg will also supply several of your classmates with enough egg white to do this miniexperiment.)

Add a few drops of the egg white to the hot water after the temperature of the water has reached 80°C.

Place 1 or 2 drops of egg white into a small beaker which contains about 3 cm^3 of 6 M HCl.

Can you explain any similarities or differences between the reaction of the egg white with the HCl and with the hot water?

B-21 Breaking the Bonds

Egg white protein behaves like many other concentrated protein solutions. There are many ionic bonds, hydrogen bonds, and hydrophobic bonds in proteins. However, these bonds are much weaker than covalent bonds. It is only because there are so many of them that the protein stays folded. If many of these weak bonds are broken, the protein will unfold.

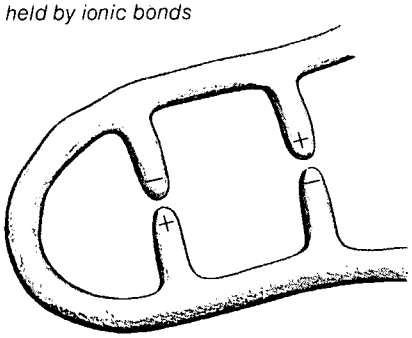
Proteins unfold at high temperatures because the weak bonds which hold the protein in its folded position are broken. No covalent bonds need to be broken to unfold the protein. In fact, you did not heat the egg white long enough or to a high enough temperature to break any of its covalent bonds. But why do the weak bonds break at high temperatures? The answer has to do with the motion of molecules.

The motion of a molecule increases as the temperature is raised. As a protein solution is heated, the vibration and rotation of the amino acid side chains increase. Finally the motion of the side chains becomes so great that the bonds between them are broken and the protein unfolds. None of the covalent bonds of the protein are broken. Therefore, the amino acids will still be in the same sequence in the chain, and the disulfide bridges will be intact even though the folding is disrupted.

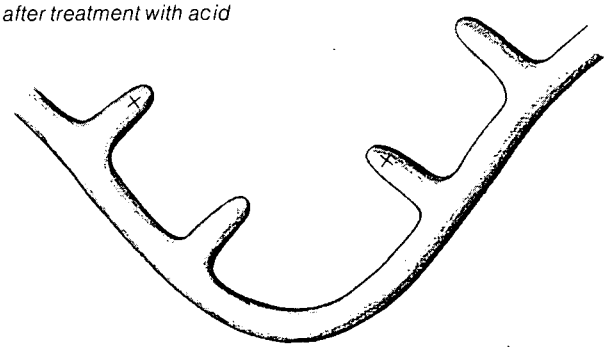
When a protein unfolds, we say that it is *denatured* because it no longer has its natural structure. This is what happened when you heated the egg. The acid in the miniexperiment also caused the egg white protein to denature. How does acid break the weak bonds that hold the protein in its folded structure? You recall that the ionic bonds were formed between the positively charged ammonium groups and the negatively charged carboxylate groups.

An egg solidifies as it is cooking because its proteins are being denatured.

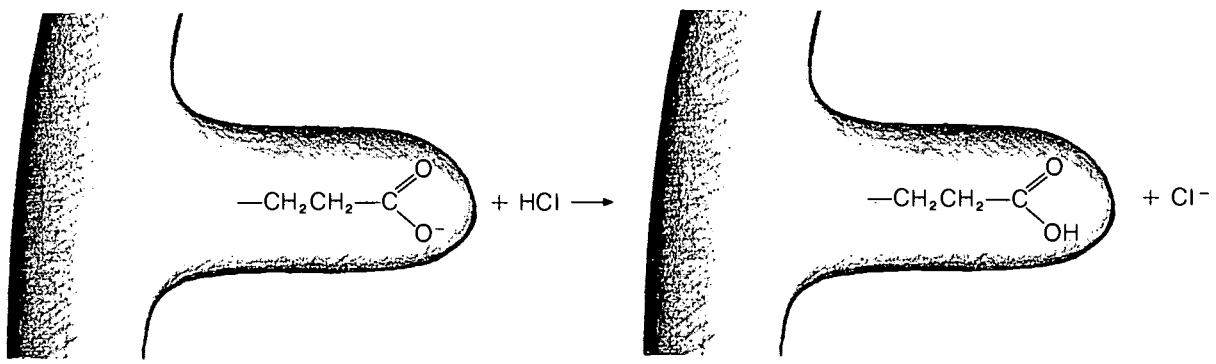
PROTEIN IN FOLDED POSITION
held by ionic bonds



UNFOLDED PROTEIN
after treatment with acid



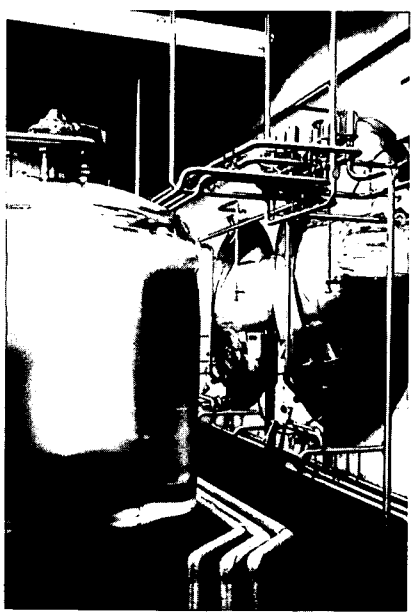
When HCl is added to the protein solution, hydrogen ions are transferred to the carboxylate groups. The negative charge on the carboxylate group is neutralized by this acid-base reaction. As a result, the only groups that still have a charge are the ammonium groups. These are all positively charged groups, so the protein can no longer form ionic bonds. In fact, the positively charged groups repel each other and force the protein to unfold.



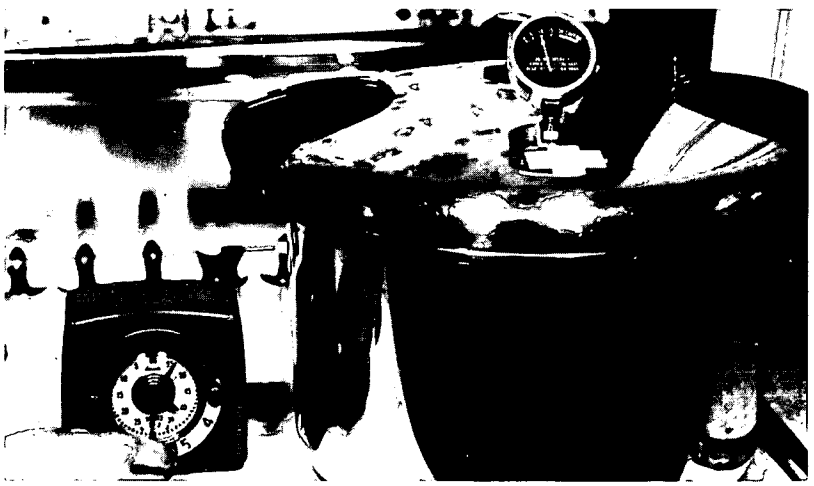
A technician uses an autoclave to sterilize equipment used in laboratory research (also see below). Items to be sterilized are sealed in the autoclave. Then, by applying heat and pressure, contaminating microorganisms such as bacteria and molds are killed.

When Louis Pasteur experimented with fermentation in wine and beer, he found that the growth of certain microorganisms could be prevented by heating the liquid to 57.2°C. This technique, called pasteurization, is applied to bottling milk, a somewhat outdated process though still in use today, (as shown below) as well as to wine and beer and even to some solid foods to retard spoilage.

We take advantage of heat or acid denaturation of proteins in many ways. For example, we sterilize surgical instruments with heat. The heat kills the bacteria by denaturing their proteins. Pasteurization of milk involves a similar process. The milk is heated just enough to denature bacterial enzymes. The protein in the milk is more stable and does not denature unless the milk is heated too much. If the temperature does get too high, the milk proteins denature and the milk curdles.



Food is sterilized during canning by cooking it in a pressure cooker. If it is not cooked well enough, bacteria will grow in the can and spoil the food. You may have read about cases in which the government ordered a manufacturer to recall millions of cans of food because the food could cause botulism. *Botulism* is a form of food poisoning that is often fatal. It is caused by a *toxin* produced by bacteria that grow in canned food that was not properly sterilized. The botulism toxin is the most poisonous compound known. One milligram of the toxin (about the mass of a few grains of salt) is enough to kill twenty million mice.



The toxic bacterium *C. botulinum* (*above*) is activated when certain foods are improperly canned. Home canners (*left*) use a pressure cooker to produce a high degree of heat to render the bacterium inactive. The food is then sealed in airtight jars. The discovery of canning is commemorated on the stamp below.

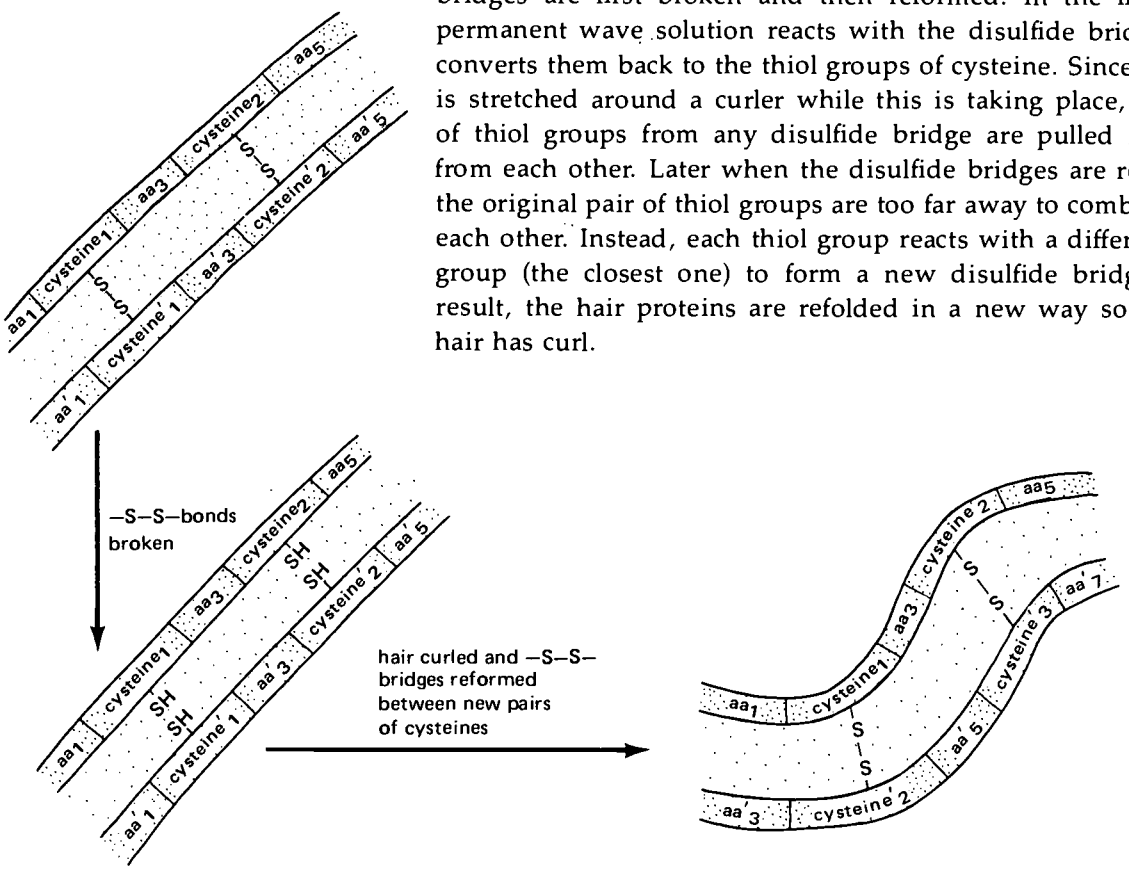
Fortunately, cases of botulism are rare. One reason is that botulism toxin is a protein that is easily denatured. Thus, if canned food is cooked before it is eaten, the poisonous properties of the botulism toxin are destroyed. In fact, most cases of botulism occur when people eat canned food without heating it.

In all of these cases, the proteins unfolded because the ionic, hydrogen, and hydrophobic bonds were broken. But there is one important case in which people change the folding of proteins by breaking disulfide bridges.

TIME MACHINE

1806	Construction begins on Cumberland National Pike, the first federal highway.
1807	Robert Fulton takes his first steamboat, *Clermont*, on a trial run up the Hudson River.
1809	Washington Irving publishes *Rip Van Winkle*.
1810	**Nicolas Appert publishes his findings on "canning" food in hermetically sealed glass containers.**
1812	Anthracite coal is first used commercially.
1813	The waltz becomes popular in Europe.

Hair is made of protein which contains a large amount of cysteine. The thiol groups (—SH) of cysteine are connected as disulfide bridges and hold the hair in its normal structure. When hair is curled by using permanent wave solutions, these disulfide bridges are first broken and then reformed. In the first step, permanent wave solution reacts with the disulfide bridges and converts them back to the thiol groups of cysteine. Since the hair is stretched around a curler while this is taking place, the pair of thiol groups from any disulfide bridge are pulled far away from each other. Later when the disulfide bridges are reformed, the original pair of thiol groups are too far away to combine with each other. Instead, each thiol group reacts with a different thiol group (the closest one) to form a new disulfide bridge. As a result, the hair proteins are refolded in a new way so that the hair has curl.



EXERCISES

1. Look at Appendix II which illustrates the structures of the common amino acids. Using a different amino acid for each example, choose an amino acid that has side chains that can participate in the following:
   a. hydrophobic bonding
   b. ionic bonding
   c. disulfide bridge formation
   d. hydrogen bonding
2. Explain what happens to a protein when it is denatured. Give examples that illustrate the importance of protein denaturation in everyday life.

B-22 pH: The Power of Hydrogen Ions

In the previous miniexperiment you discovered that protein was denatured when it was added to 6 M HCl. Do you think egg protein would be denatured if it were added to an extremely dilute solution of hydrochloric acid—say 0.0001 M HCl? The answer is no. It would not denature. Obviously, the concentration of acid present in solution is important.

The acidities of various biological fluids are often different. You may know that stomach fluid is acidic but that blood is not. The lining of the stomach secretes many substances, including the enzyme pepsin and hydrochloric acid. The hydrochloric acid adjusts the pH of the stomach fluids to a level that makes it possible for the pepsin to function.

The pH of a solution represents its acidity or alkalinity. Examine the pH scale in the following table. You can see that pH is a measure of the H^+ concentration. As the pH becomes greater, the H^+ concentration becomes less. A change of 1 pH unit means the H^+ concentration has changed by a factor of 10. For example, the concentration of H^+ at pH 3 is ten times greater than the concentration of H^+ at pH 4. The pH scale is a convenient way of expressing a wide range of H^+ concentrations.

It is an important fact that water always contains both H^+ and OH^- ions. This is because water itself can *dissociate* (come apart).

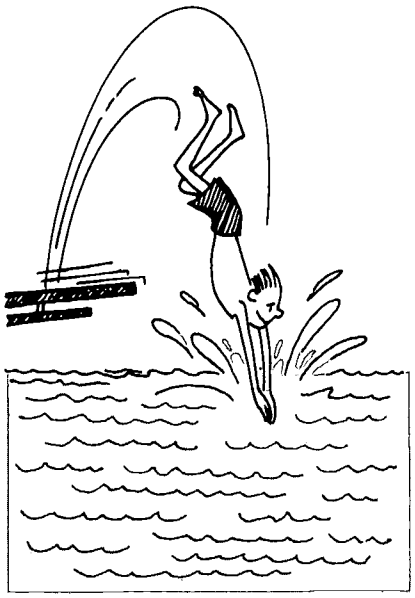
$$H_2O \longrightarrow H^+ + OH^-$$

When water molecules dissociate, equal amounts of H^+ and OH^- are formed. Thus, in pure water the H^+ concentration is equal to the OH^- concentration.

The H^+ concentration of pure water is 10^{-7} M. Thus, pure water has a pH of 7. You can see that the amount of H^+ in pure

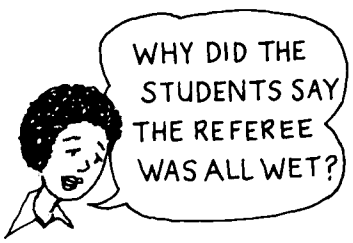
TIME MACHINE

1906	O. Henry's *The Gift of the Magi*, is published.
1907	Pablo Picasso paints *Les Demoiselles d'Avignon*—his first Cubist work.
1908	Henry Ford produces the first Model T automobiles.
1909	**Danish biochemist Søren Sørensen introduces the concept of pH.**
1909	Danish botanist Wilhelm Johannsen first uses the term *gene*.
1910	Leo Baekeland manufactures first completely synthetic plastic, later known as Bakelite.
1911	Irving Berlin composes "Alexander's Ragtime Band."



THE pH SCALE

H^+ (mole/liter)		pH		OH^- (mole/liter)
(1)	10^0	0		10^{-14}
(0.1)	10^{-1}	1		10^{-13}
(0.01)	10^{-2}	2		10^{-12}
(0.001)	10^{-3}	3		10^{-11}
(0.0001)	10^{-4}	4		10^{-10}
	10^{-5}	5		10^{-9}
	10^{-6}	6		10^{-8}
	10^{-7}	7	**PURE WATER**	10^{-7}
	10^{-8}	8	**(neutral)**	10^{-6}
	10^{-9}	9		10^{-5}
	10^{-10}	10		10^{-4}
	10^{-11}	11		10^{-3}
	10^{-12}	12		10^{-2}
	10^{-13}	13		10^{-1}
	10^{-14}	14		10^0



water is small and, therefore, that only a small number of water molecules actually dissociate. In fact only one water molecule out of about five-hundred-fifty million normally dissociates.

When the H^+ concentration of a solution is greater than the OH^- concentration, the solution is *acidic*. When the H^+ concentration is less than the OH^- concentration, the solution is *basic*. In pure water the concentration of H^+ is the same as the concentration of OH^-; so the solution is neither acidic nor basic. It is *neutral*.

EXERCISES

EXAMPLES:

1. What is the pH of a 0.01 M HCl solution? In order to calculate this, you need to remember that HCl dissociates when it is dissolved in water. The HCl is converted completely to H^+ and Cl^-; so the H^+ concentration is 0.01 M. From the pH table you can see that when the H^+ concentration is 0.01 mole/liter the pH is 2.
2. If the pH of a solution is 4, what is the H^+ concentration? From the table you can see that the concentration of H^+ at pH 4 is 0.0001 M (10^{-4} M).

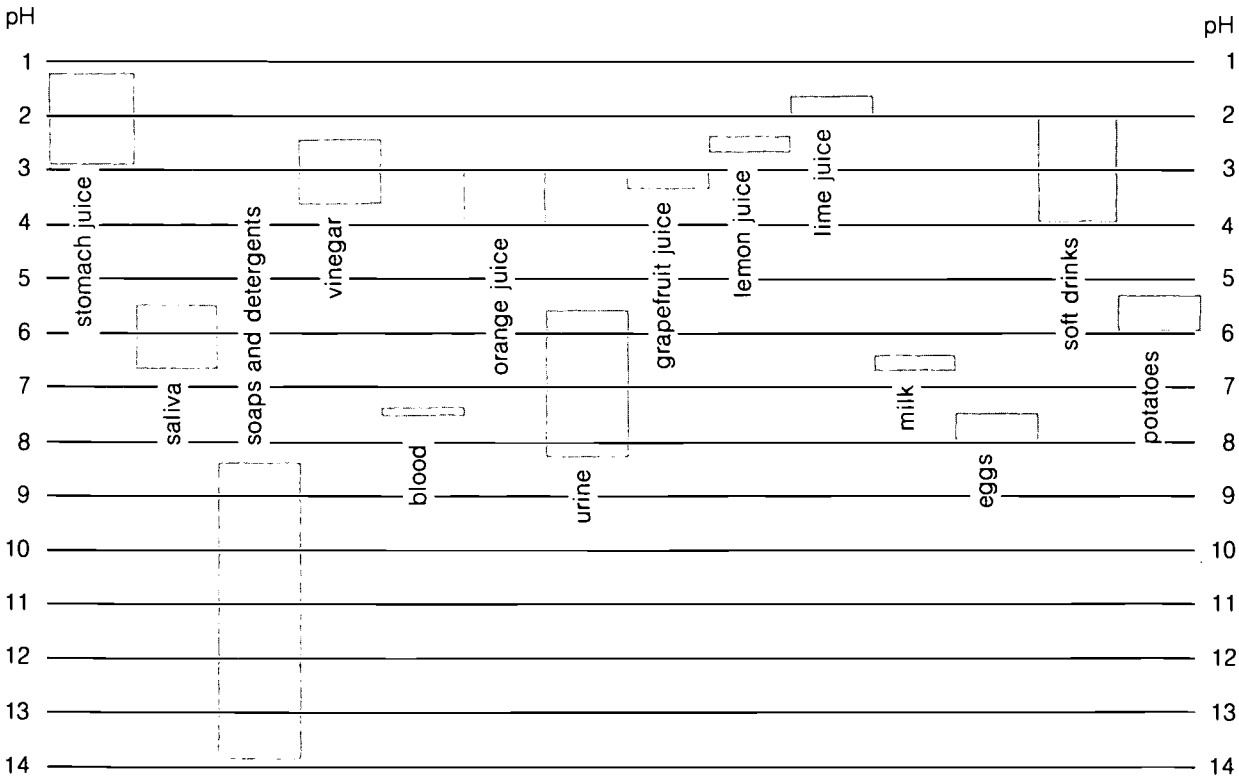
NOW TRY THESE:

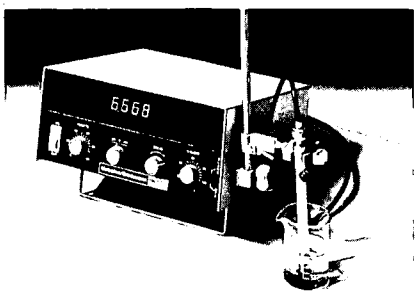
1. What is the pH of a 0.1 M solution of HCl?
2. What is the H^+ concentration if the pH is 9?
3. How many times more concentrated is the H^+ in a solution that has a pH of 1 than a solution that has a pH of 7?

4. What is the pH of a solution of 0.1 M NaOH? We can do this because when sodium hydroxide dissolves, it is completely dissociated into sodium (Na^+) and hydroxide (OH^-) ions.
5. What is the pH of a solution containing 3.65 grams (g) of HCl per 100 cm^3?
6. Would you describe a solution that has a pH of 5 as acidic, basic, or neutral?

The pH of biological fluids is significant. The pH of a number of common solutions is listed in the following table. You can see that the juice of the stomach is acidic. The pH can vary from about 1 to 3. The rest of the body fluids have a pH near 7. They are almost neutral solutions. The pH of the blood is constant. It is normally about 7.4. This value is critical. If a disease such as diabetes causes the blood pH to drop as low as 7.0 the patient may die. The acids that cause the blood pH to decrease in diabetes are produced by metabolism when there is too little insulin in the blood.

THE pH OF SOME COMMON SUBSTANCES





Modern laboratories are equipped with electronic instruments that are able to measure the pH of solutions. Here the pH of a liquid is being measured by a pH meter and displayed to several decimal places.

Unlike blood, the pH of urine can vary over a wide range. It can be as low as 5 or as high as 8. The exact pH of the urine will depend on what you have recently eaten and on your health. If you eat large amounts of certain foods, such as most vegetables and fruits, your urine will tend to have a more basic (higher) pH. On the other hand, if you eat a large amount of high protein food, your urine will have a more acidic (lower) pH. The reason for this is that excretion products from the metabolism of fruits and vegetables are basic, whereas the excretion products of protein metabolism are acidic.

EXERCISES

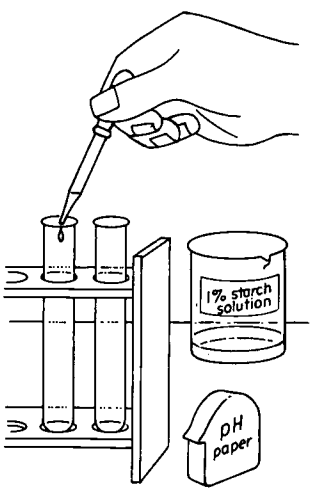
The lining of the stomach secretes HCl to produce stomach acid.

1. If the pH of the stomach juice is 3, what is the H^+ concentration?
2. If the pH of the stomach juice is 1, what is the H^+ concentration?
3. How many moles of HCl are there in 100 cm^3 of stomach juice that has a pH of 1?
4. How many grams of HCl are there in the solution in part 3?

Right about now you are probably saying to yourself, "What does all this business about pH have to do with enzymes?" Good question! The pH of an enzyme solution is very important. Some enzymes work best in acid conditions (low pH), some work best at neutral pH, and some work best at basic (high) pH.

miniexperiment

B-23 Inspecting the Expectorate



Saliva contains an enzyme called *amylase* which digests starch. Amylase is active at the pH in the mouth (almost neutral).

Add 10 drops of 1-percent starch solution to each of two test tubes. Add 2 drops dilute (2 M) HCl to one of the tubes. Collect some saliva in a tube and add 2 to 3 drops to each of the tubes containing the starch.

Test the pH in each test tube with universal pH paper. Wait 10 minutes. Add 1 drop of starch-iodine test solution to each tube.

Do you think the amylase present in saliva is active in the stomach after the food is swallowed? Why?

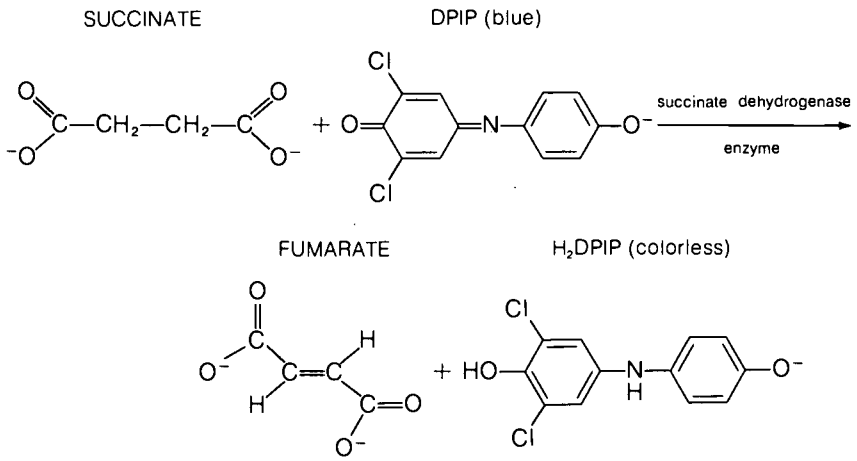
You should not get the idea that all enzymes are inactive at the pH of the stomach. The digestive enzyme pepsin, which hydrolyzes proteins, is actually found in the stomach juice. It works best at about pH 2 and is completely inactive at neutral pH. However, it is a somewhat unusual enzyme. Most enzymes in your body have their maximum activity near neutral pH.

B-24 Succinate Dehydrogenase

The results of the amylase experiment show that large changes in H^+ concentration cause large changes in enzyme activity. You probably would have predicted that from the results of the experiment with the egg protein. But what about enzyme activity at pH 5 or pH 9? These solutions are not neutral, but they are not strongly acidic or strongly basic either.

In order to answer this question, you need to be able to measure quantitatively the amount of enzyme activity. You will do this in the next experiment by measuring the rate of the reaction catalyzed by *succinate dehydrogenase*. This enzyme is part of the metabolic pathway that oxidizes carbohydrates and fats to water and carbon dioxide. One of the functions of this pathway is to provide metabolic energy so the cell can carry out energy-requiring processes. Thus, this enzyme is found in all tissues, but it occurs in especially high concentrations in tissues that do large amounts of work, such as the heart.

The equation for the reaction you will observe is:



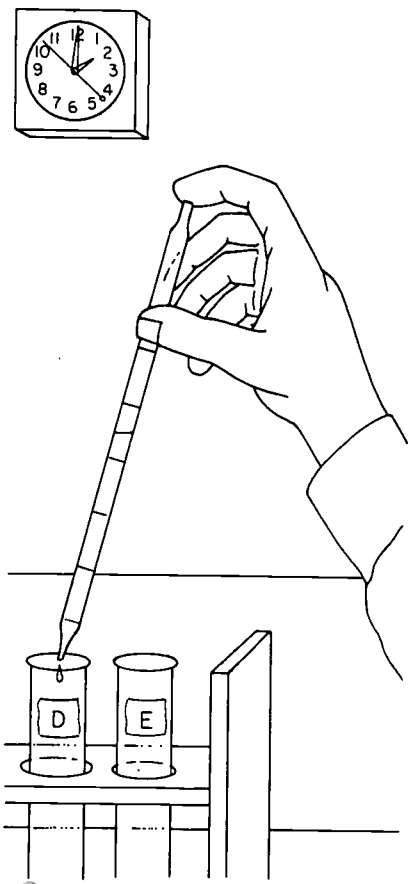
The enzyme is called *succinate dehydrogenase* because it catalyzes the removal of two hydrogen atoms from succinate—that is, it dehydrogenates succinate. These hydrogen atoms are transferred to DPIP. The chemical name for DPIP is 2,6-*d*ichloro*p*henol*i*ndo-*p*henol. Anybody would rather call it DPIP!

The reaction you will observe in the laboratory is not exactly the same as the reaction that the enzyme catalyzes in the cell because cells do not contain DPIP. We are using DPIP as an artificial reactant for the strictly practical reason that we can observe the disappearance of the blue color. The blue DPIP is converted to the colorless H_2DPIP as the reaction proceeds. This lets us quantitatively measure the rate of the enzyme reaction.

As we explained earlier in this module, the rate of a reaction is analogous to the speed of a car. It is simply the number of moles that reacts in a given length of time. You can express the speed of a car in kilometers (km) per hour and the reaction rate in moles per minute. For example, suppose 0.6 mole of DPIP was used up in 2 minutes in the preceding succinate dehydrogenase reaction. The reaction rate would be 0.6 mole/2 minutes = 0.3 mole/minute.

EXPERIMENT

B-25 pH and Succinate Dehydrogenase



Set up a rack of test tubes labeled A, B, C, D, and E. Fill the test tubes with the following amounts of solution.

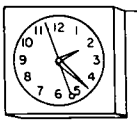
Test tube	Volume and pH of buffer	Volume of 0.1 M succinate (cm^3)	Volume of enzyme (cm^3)
A*	5 cm^3—pH 7.3	1	1
B	5 cm^3—pH 6.3	1	1
C	5 cm^3—pH 7.3	1	1
D	5 cm^3—pH 8.0	1	1
E	5 cm^3—pH 12.0	1	1

*This tube does not receive any DPIP and serves only as a reference to which the other tubes are compared in order to determine when the reaction is complete. Prepare this tube first and set it aside to use for comparing colors.

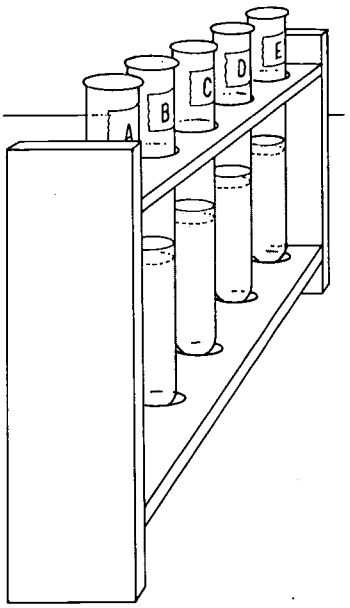
To measure the rate of the enzyme reaction, work in pairs and use the following procedure.

One partner records the time at which the reaction starts and the time at which it ends (to the second). The other partner uses a pipet to add 1 cm^3 of DPIP to test tube B. Mix immediately. Start timing as soon as the DPIP is added and mixed. Then add a dropperful of mineral oil to cover the surface of the solution.

Repeat this procedure for test tubes C, D, and E. Remember, test tube A is a blank for comparison of color or lack of color.



You will need to construct a data table similar to the one below to record your results.



Test tube label	pH	DPIP (moles)	Time at start	Time at end	Rate (moles/minute)

Now, calculate the rate of enzyme reaction in each tube. This is really very simple. All you need to know is the amount (in moles) of DPIP that is used and the number of minutes and seconds the reaction takes.

An example of the calculation follows.

1. If you add 1 cm^3 of 0.000 30 M DPIP, you would have added 0.000 000 30 or 3.0×10^{-7} moles DPIP. Remember, M means the concentration in mole/liter. (See *Reactions and Reason: An Introductory Chemistry Module.*)
2. If the clock read 2:23:15 at the start of the reaction and 2:28:30 when the reaction was completed, the time required was 5.25 minutes.
3. The rate of reaction is:

$$\frac{\text{moles DPIP}}{\text{minutes reaction}} = \frac{3.0 \times 10^{-7} \text{ moles}}{5.25 \text{ minutes}} = 5.7 \times 10^{-8} \text{ moles/minute}$$

MOLE

Make a graph of your results to show the influence of pH on the reaction rate. Plot pH on the *x*-axis and reaction rate on the *y*-axis. Connect the points with a line. Over what pH range is this enzyme active?

MILLIMOLE

The results you obtained in this experiment are very awkward to use because they are such small numbers.

Fortunately, the metric system provides a simple way to convert data to more convenient numbers. Convert the reaction rates that you found to the following:

MICROMOLE

a. millimoles/minute (mmole/minute) 1 mmole = 10^{-3} mole
b. micromoles/minute (μmole/minute) 1 μmole = 10^{-6} mole
c. nanomoles/minute (nmole/minute) 1 nmole = 10^{-9} mole

NANOMOLE

TIME MACHINE

1834	Jacob Perkins patents an ice-making machine.
1835	**Jöns J. Berzelius introduces term "catalysis" for process by which a substance brings about chemical reactions in other substances without itself being consumed in the reaction.**
1836	First and Second McGuffey Readers are published, first in a series that will sell more than 122 million copies.
1837	Blacksmith John Deere makes the first Grand Detour steel plow.
1837	First kindergarten opens, in Germany.
1838	Charles Dickens's *Oliver Twist* is year's bestseller.
1839	Edgar Allan Poe publishes "The Fall of the House of Usher."

B-26 The Potent Part of the Protein

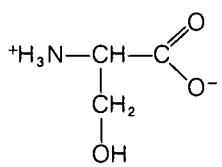
We have found that many of the properties of enzymes can be explained in terms of protein structure. For example, heat and acid destroy enzyme activity by denaturing the enzyme. But how does an enzyme catalyze a reaction? What is responsible for the high degree of *specificity* of enzyme reactions? These are questions we will consider in this section.

Catalysis of a reaction does not occur at just any random place on the surface of an enzyme. Instead, it takes place in a specific region called the *active site*. The active site is not only responsible for the catalytic activity of the enzyme but also for the specificity of the enzyme. Thus, understanding the active site is crucial to understanding enzymes.

The active site of an enzyme that catalyzes one reaction is different from the active site of an enzyme that carries out a different reaction. All active sites, however, do have some properties in common. For example, side chains of amino acids are important in the reactions at the active sites of all enzymes.

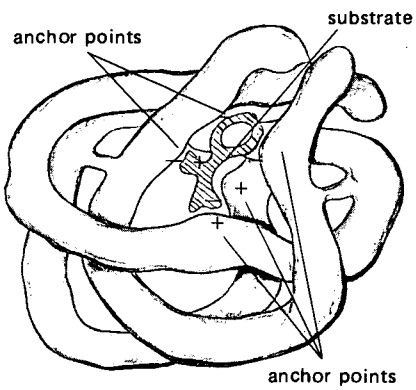
Some side chains form bonds with the reactant and serve as *anchor points* to hold the reactant in place in the active site. Two types of anchor-point bonds are the ionic bond and the hydrogen bond. These are the same types of bonds that help keep the folds of the protein in place.

SERINE



How do the side chains of the anchor-point amino acids form bonds with the reactant and hold it in place? Suppose an enzyme catalyzes a reaction of the amino acid *serine*. Serine has an ammonium group that has a positive charge and a carboxyl group that carries a negative charge. These groups are able to form ionic bonds. Serine also has a hydroxyl group that is able to form hydrogen bonds. If the active site of the enzyme contains side chains with appropriate functional groups in the proper positions, the serine molecule will be able to fit snugly in the active site.

ENZYME



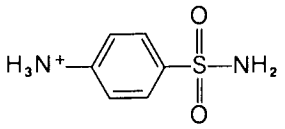
However, other molecules that have the same functional groups as serine will not fit as well. You will be able to observe this more clearly in the following illustration, *Substrate and a Similar Molecule in the Enzyme Active Site*. You can see that the anchor-point side chains are in good positions to form bonds with the functional groups of the serine molecule. However, the other molecule with the same functional groups as serine does not fit the active site as well. Thus, the enzyme will only be able to hold and catalyze the reaction with serine.

This explains enzyme specificity; the anchor points in the active site are exactly positioned so that the true *substrate* fits best and therefore reacts best. A substrate is the substance the enzyme

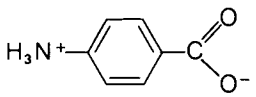
acts on. Of course, other molecules may also fit in the active site but usually not as well as the true substrate.

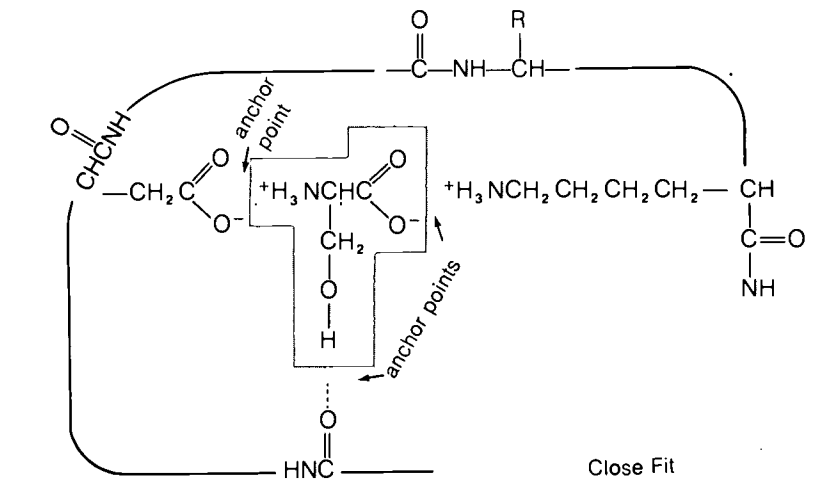
It is sometimes possible to find molecules that will fit the active site but that cannot react. Of course, when one of these unreactive compounds is in the active site, the true substrate will not be able to enter the site. These unreactive compounds act as *enzyme inhibitors* by filling the active site on an enzyme molecule and preventing the enzyme from catalyzing the reaction of the true substrate. This is the basis of the action of many (but not all) drugs. They act as enzyme inhibitors by filling the active site. An example of an antibiotic that acts this way is sulfanilamide. The structure of sulfanilamide is similar to that of aminobenzoic acid, an important compound in the metabolism of some kinds of bacteria. Sulfanilamide can inhibit an enzyme that normally catalyzes a reaction of aminobenzoic acid. It does this by filling the active site. Since the bacteria cannot multiply unless they can carry out the aminobenzoic acid reaction, the sulfanilamide prevents their growth.

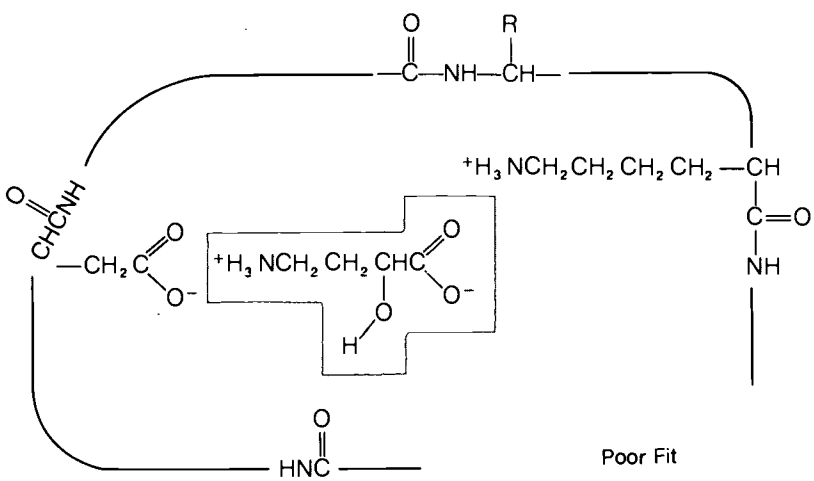
SULFANILAMIDE



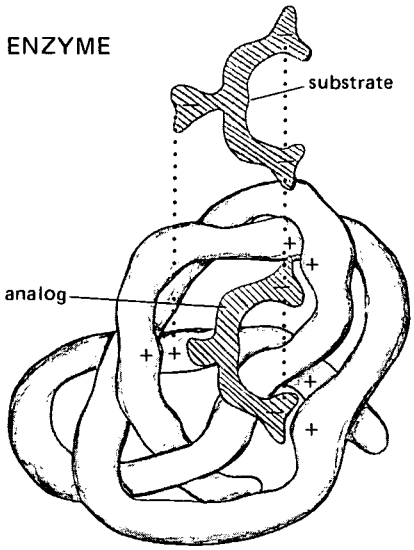
AMINOBENZOIC ACID







The top figure shows the close fit between the substrate and the anchor points of the enzyme. The figure on the bottom shows that a small molecule having the same functional groups as the true substrate will not fit as well if the functional groups do not match up with the anchor points.



Biochemists have developed a number of ways to determine important characteristics of the active site. In one of these methods, they take advantage of the fact that the substrate must bind to the active site before the reaction can be catalyzed. They do this by studying the effects of substrate analogs on the rate of the enzyme reaction. A *substrate analog* is a compound which has a structure similar to that of the true substrate. Very often the substrate analog will be able to bond to the enzyme anchor points just like the true substrate, but it may not be able to undergo the chemical reaction. If it does bind in the site, it will prevent the true substrate from entering the site and will act as an enzyme inhibitor.

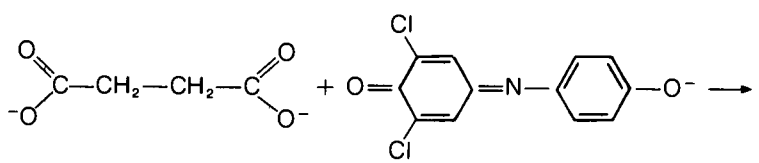
Not all substrate analogs will bind in the active site even though they may appear to be quite similar to the true substrate. A functional group necessary for bonding with an anchor point may be missing from the analog, or the functional groups may be in the wrong position to fit snugly in the active site. Those that do not bind in the active site will not usually interfere with the reaction. We can learn a great deal about the active site by determining which substrate analogs bind in the site. Analogs that do bind will inhibit the enzyme.

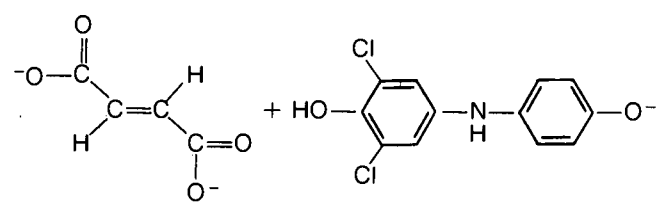
In the next experiment you will investigate the influence of substrate analogs on the rate of the reaction catalyzed by succinate dehydrogenase. You will use two substrate analogs, namely malonate and propionate. Compare their structures with the structure of the true substrate, succinate.

EXPERIMENT

B-27 The Active Site

In section *B-24* we discussed the succinate dehydrogenase reaction. You will recall that succinate is converted to fumarate in this reaction.

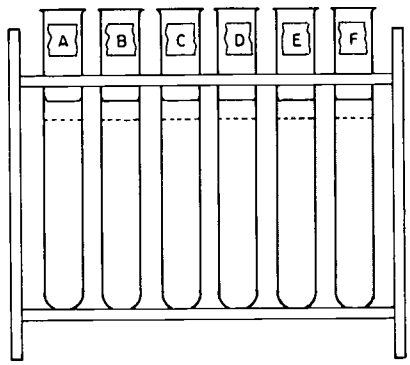




As in experiment *B-24*, to measure the rate of the enzyme reaction we will take advantage of the fact that the blue DPIP is converted to the colorless H_2DPIP.

Label and fill six test tubes according to the following scheme.

Tube	Buffer cm^3	Enzyme cm^3	Succinate cm^3	Malonate cm^3	Propionate cm^3	Water cm^3
A*	5	1	0	0	0	2
B	5	1	1	0	0	1
C	5	1	1	1	0	0
D	5	1	1	0	1	0
E	5	1	0	1	0	1
F	5	1	0	0	1	1



* This tube does *not* receive any DPIP and serves only as a reference to which the other tubes are compared in order to determine when the reaction is complete.

To measure the rates of the enzyme reactions, one partner watches the clock and records the time at which the reaction starts and the time at which it ends—to the second. (Start timing as soon as the DPIP is added and mixed.)

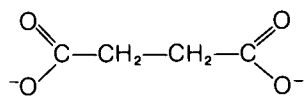
Using a pipet, the other partner adds 1 cm^3 of DPIP to test tube B. Mix immediately. Add an eyedropperful of mineral oil to cover the surface of the solution. Repeat this procedure for test tubes C, D, E, and F.

The reaction in some test tubes may be slow. If you cannot see a change after 4 minutes, carry out the procedure with the next test tube.

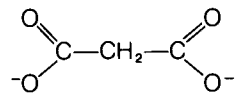
Make a data table with the following column headings and use it to record your data.

Tube	Compound present	Time at start	Time at end	Reaction (minutes)	Reaction rate

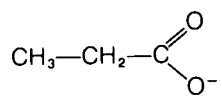
SUCCINATE



MALONATE



PROPIONATE

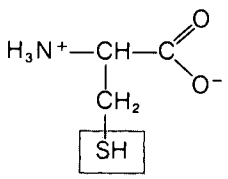


Questions:

1. Did succinate dehydrogenase catalyze the reaction of malonate and propionate with DPIP?
2. Does malonate bind in the active site?
3. Does propionate bind in the active site?

4. Is one carboxyl group enough for an analog to bind in the active site?
5. The active site must contain anchor points. What functional groups in the protein could act as anchor points? How many must there be?
6. Assume that propionate could bind to the active site. Could the enzyme dehydrogenate propionate? What would the product be?
7. Look at the structures of succinate, fumarate, and malonate. Explain why the enzyme cannot dehydrogenate malonate.

CYSTEINE



thiol group

B-28 Other Features of the Active Site

Not all the amino acid side chains in the active site serve as anchor points. Some of the side chains actually participate directly in the chemical reaction. Different side chains are involved in the reaction at the active sites of different enzymes. One side chain that is often (but not always) involved in the reaction at the active site is the side chain of cysteine. It contains a thiol group.

For example, the thiol group is known to be involved in the reaction of the active site of papain, an enzyme obtained from papayas. Actually, you may have used this enzyme yourself since it is the active ingredient in some brands of meat tenderizer. Papain catalyzes a reaction of the peptide bonds of proteins and breaks them down into smaller proteins and ultimately into amino acids. This is exactly what the papain in meat tenderizer does to meat: it degrades the proteins that cause the meat to be tough.

The fact that —SH groups can sometimes participate in reactions at active sites helps to explain why some metal ions such as mercury, lead, and silver are poisonous. These ions react strongly with —SH groups to produce stable compounds.

$$R—S—H + HgCl_2 \longrightarrow R—S—Hg—Cl + HCl$$

When a mercury(II) ion reacts with a thiol group that is an essential part of an active site, the enzyme is inactivated. Very little mercury is required to inhibit enzymes containing —SH groups in the active site. If a small fraction of the enzymes in the body are inactivated, the effects may not be noticeable. However, if a substantial fraction of the —SH containing enzymes are inactivated, metabolism will be upset and symptoms of mercury poisoning will appear.

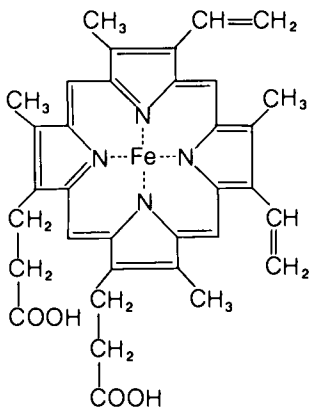
The side chains of amino acids are the only reactive groups in the active sites of some enzymes. However, you should not get the idea that all enzymes are made only of protein or that the only groups at the active site are the side chains of amino acids. Nothing could be further from the truth. The active sites of many enzymes contain other kinds of important reactive molecules in addition to amino acids.

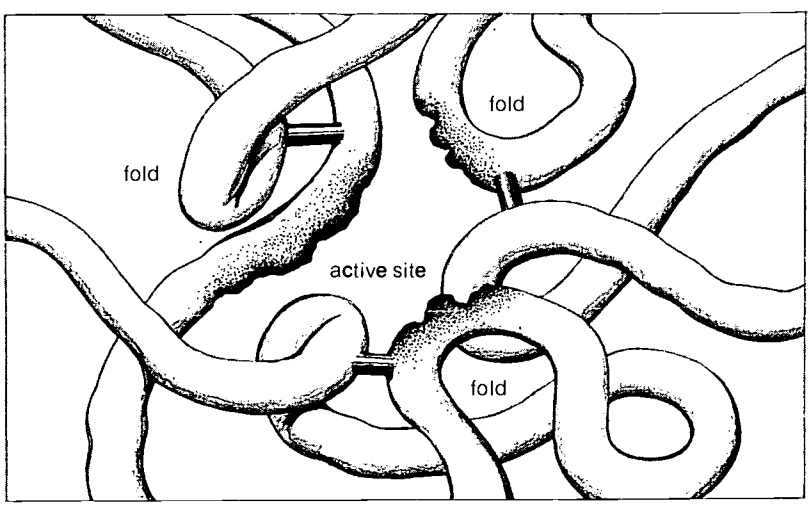
One well-known example is the iron-containing compound *heme*. It serves as part of the active site of *hemoglobin*, which carries oxygen in the blood. Heme is also part of the active site of catalase, which decomposes hydrogen peroxide. The enzyme succinate dehydrogenase has the vitamin, riboflavin, at its active site. Other enzymes contain other organic compounds and even simple metal ions, such as Cu^{2+}, at their active sites. All of these substances participate in the reaction at the active site and thus are essential for enzyme activity. This is why you must have minerals and vitamins in your diet. Enzymes could not work if they were absent.

In order for a reaction to occur at the active site, all the components of the site must be held close together in precise positions. For example, amino acid side chains that serve as anchor points must be properly positioned so that the substrate molecule can bind to the active site. However, the amino acids at the active site are not necessarily neighbors of each other in the sequence of amino acids making up the protein chain. In fact there can be dozens of amino acids separating them in the sequence. They are held in the correct position near each other in the active site by the folding of the protein.

Now you can see how denaturing an enzyme can affect the reaction at the active site. If the folding is disrupted by extreme pH or high temperature, the amino acid side chains in the active

HEME





Some Active Sites Contain:

Fe^{2+}
Fe^{3+}
Cu^{2+}
Zn^{2+}
Mn^{2+}
$Mo^{?+}$

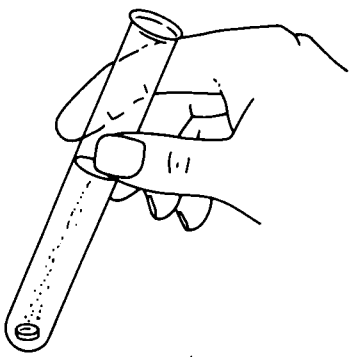
The folding of the protein places amino acids near one another at the active site in an enzyme. If the protein chain were drawn out, the amino acids in all likelihood would be widely separated. The amino acids at the active site are not necessarily sequential neighbors in the chain.

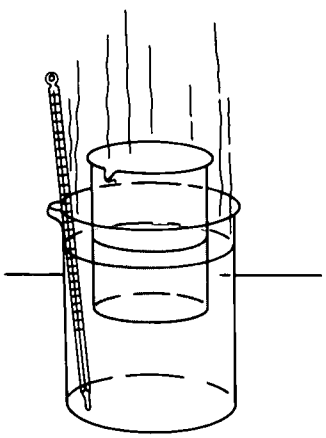
site will be moved from their critical positions. When this happens, the enzyme will lose its ability to bind the substrate and thus lose its ability to catalyze reactions. But suppose we had not raised the temperature enough to denature the enzyme. What would have happened to the reaction rate as the temperature increased? This is the question we will investigate in the next experiment.

In the following experiment you will determine the effect of temperature on the rate of an enzyme-catalyzed reaction. The reaction involves the formation of a curd (protein precipitate) from milk. The curd consists primarily of milk proteins that are clumped together. The major protein in the curd is called *casein*. This curd formation is brought about by the action of an enzyme that hydrolyzes one of the peptide bonds in casein and removes a relatively small, hydrophilic piece of protein. When this happens, the rest of the casein becomes insoluble and precipitates as a curd. Curd formation is the first step in making cheese. The enzyme you will use is called *rennin*. It is a component of an extract of calf stomach called *rennet*.

EXPERIMENT

B-29 Temperature and Reaction Rates





In this experiment, you will measure the time required for rennet to curdle milk at three temperatures: room temperature (22–25°C), 35°C, and 45°C.

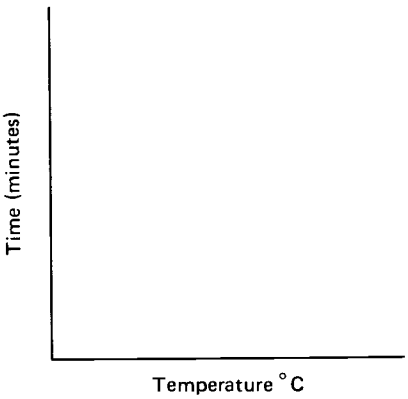
Place one rennet tablet in a test tube containing 5 cm^3 of water. The rennet will *not dissolve* but will result in a turbid suspension. *Do not heat the rennet suspension.*

Heat 120 cm^3 of skim milk in a 250-cm^3 beaker to 45°C. *Stir constantly to avoid scalding the milk.* Pour one-third of the heated milk into each of three 150-cm^3 beakers. Place one beaker on the laboratory bench and allow it to cool to room temperature.

Place the second beaker inside a larger beaker containing warm (35°C) water. Place the third beaker inside a larger beaker containing 45°C water.

When the milk at 45°C has reached a steady temperature, add 1 cm^3 of rennet suspension to it and stir. Place the beaker containing the milk and rennet suspension back in the water bath immediately after adding the enzyme. Record the time it takes from the addition of the rennet to the first visible evidence of curd formation. Repeat this procedure with the milk in the other two beakers after they have reached a steady temperature.

Make a graph of your results. Label the *x*-axis with the temperature of the reaction and the *y*-axis with the reaction time observed.



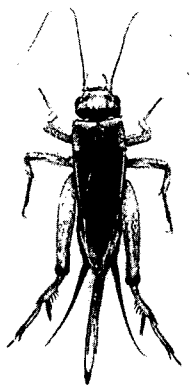
Questions:

1. Rennet contains the enzyme rennin. What experiment would you do to show that the reaction you observed was due to enzymatic activity in the rennet preparation?
2. We have said that the curd is a protein precipitate. What tests could you perform to determine if this statement is true?
3. Suppose you had cooled some of your milk on ice or in the refrigerator. What effect would this have on the rate of the curd formation catalyzed by rennin?

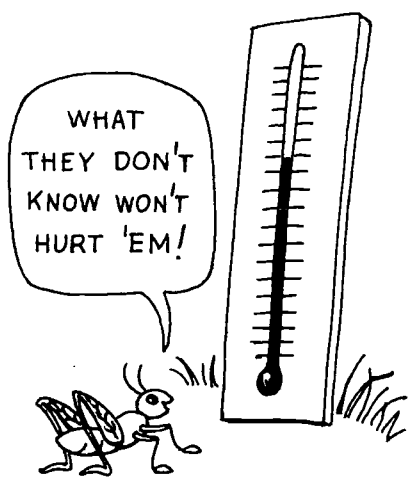
Every day you take advantage of the fact that reactions are slow at cold temperatures when you store food in the refrigerator. The enzymes in the molds and bacteria that spoil the food are slowed down by the cold. Thus, the food does not spoil as fast as it would at room temperature. Of course, the enzymes are not completely stopped; so even refrigerated food will spoil if it is stored too long.

Most of the enzymes in your body are not affected by changes in the temperature of your environment. This is because your body temperature is kept constant by metabolism at about 37°C. But have you ever noticed that your hands seem to get "stiff" when they get cold? The reason is that the enzyme reactions that cause the muscles to contract slow down when your hands are cold. As a result, you cannot contract your muscles as fast and your hands seem stiff.

Of course, cold-blooded animals such as snakes, frogs, and insects are greatly influenced by environmental temperature. They become sluggish when they are cold. The reason is the same. The enzyme reactions responsible for their activity are slower at low temperatures, and the animals cannot move as fast as they can when they are warm. Biologists have used these facts as the basis of an ingenious way to estimate temperature. They have found that the number of chirps that a cricket makes in a minute depends upon the temperature. The lower the temperature, the fewer are the chirps per minute. The equation by which you can calculate the temperature is $T(°C) = 3 + 5C/9$ (C = number chirps in 15 seconds). This particular equation works only for one species of cricket. If you happen to try it with another species, you may get the wrong answer. If you do, you can always "calibrate" your cricket.



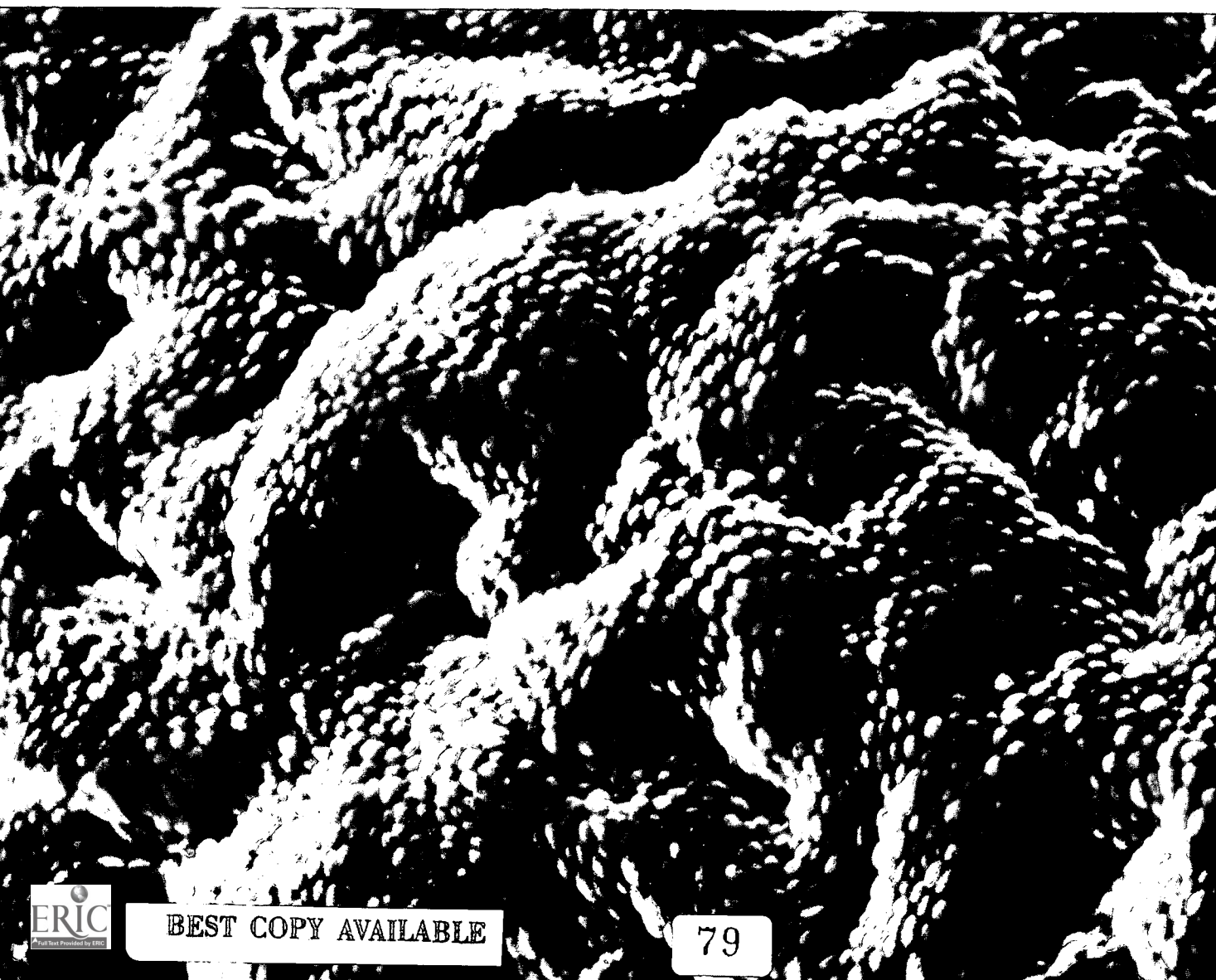
Only the adult male cricket produces the chirping sound. He does this by rubbing his forewings together.



Metabolism: The Community of Enzyme Reactions

Enzymes are an essential part of the biochemical process known as metabolism. *Metabolism* consists of all the chemical transformations that occur within an organism. Metabolism includes the reactions that break down carbohydrates and lipids to water and carbon dioxide to produce energy. It also includes all the reactions by which compounds such as proteins, lipids, and carbohydrates are synthesized.

Cells of the stomach lining appear as knobs covering the folds of the stomach in this photomicrograph (magnified 400 times). Whenever you eat, the cells of the stomach lining secrete hydrochloric acid to aid digestion.



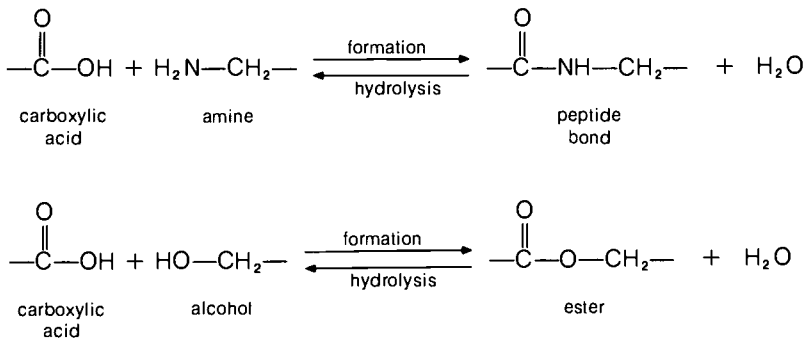
B-30 Digestion: The First Step of Metabolism

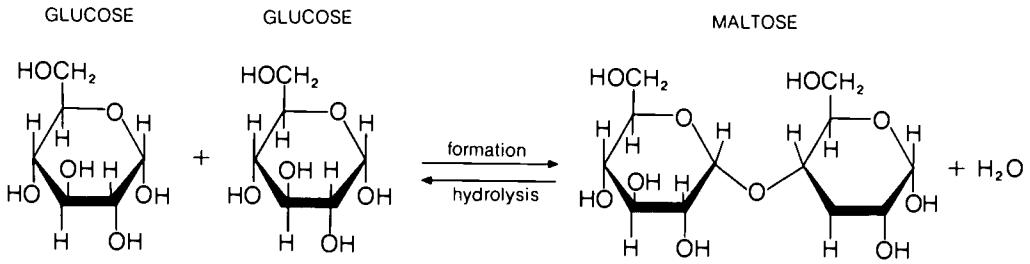
There are so many metabolic reactions that we still do not know all of them, and a great deal of scientific work remains to be done. However, thousands of metabolic reactions have been discovered. What we do know about metabolism is so great that we can only investigate a very small part of it in this module. We will focus our attention on the reactions of digestion and carbohydrate metabolism because these reactions are among the most important in your body.

You know that all compounds in your body either come directly from your food or are made from compounds you get in your food. However, the substances contained in food are not ready to be used by the cells in your body. In fact, most food would be extremely poisonous if you injected it directly into your bloodstream. Food must first be converted into a form that your body can use. This job is performed by the enzymes in the digestive tract.

There are a large number of enzymes in the digestive tract. Some of them are present in saliva, some are contained in stomach juice, and some are found in the small intestine. All of these enzymes serve to break down the materials in your food into smaller molecules that your body can use. Some of the enzymes catalyze the breakdown of carbohydrates, and some break down nucleic acids such as RNA. (Structures of nucleic acids are discussed in section *B-49*.) Other digestive enzymes break down proteins or lipids. You might suppose that the chemistry of digestion would be very complicated since there are so many enzymes catalyzing the breakdown of so many types of compounds. But actually digestion is simple. Chemically speaking, all of these enzymes catalyze one type of reaction called *hydrolysis*.

What is the *hydrolysis reaction*? You recall that water is split out when starch is formed from glucose and also when ester bonds are formed in lipids or peptide bonds are formed in proteins. These reactions are summarized in the following illustration.



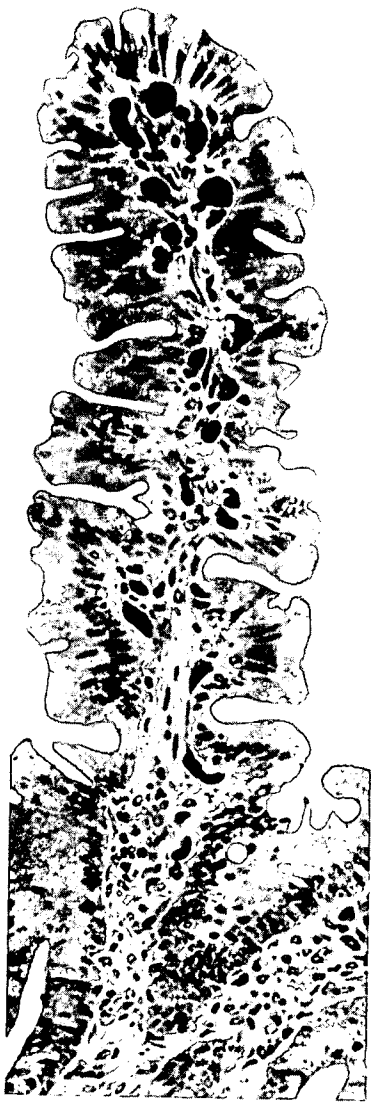


The hydrolysis reaction is just the reverse of the reactions by which the polysaccharides, esters, and peptide bonds are formed. The enzymes hydrolyze the molecules by replacing the water that was split out. Several digestive enzymes are listed in Table 3. You are probably familiar with some of these enzymes already.

TABLE 3: SOME DIGESTIVE ENZYMES

Amylase	found in saliva and the pancreatic juice secreted into the small intestine	hydrolyzes starch to the disaccharide maltose
Maltase	found in cells lining the small intestine	hydrolyzes maltose to glucose
Pepsin	found in gastric (stomach) juice	hydrolyzes peptide bonds; results in amino acids and smaller proteins
Trypsin	secreted from pancreas into small intestine	hydrolyzes peptide bonds; results in amino acids and smaller proteins
Lipase	secreted from pancreas into small intestine	hydrolyzes triglycerides to fatty acids and glycerol
Ribonuclease	secreted from pancreas into small intestine	hydrolyzes RNA to nucleotides*

*See section *B-49* for a definition of nucleotides.



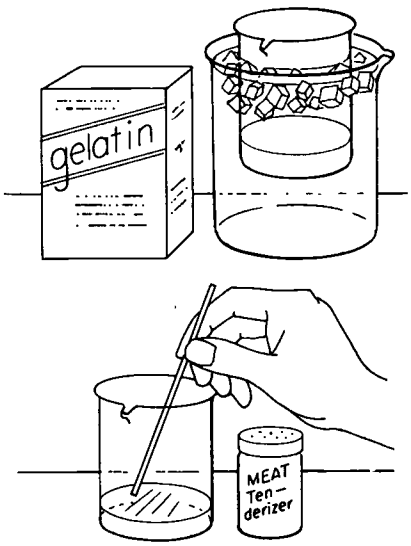
The surface of the small intestine is covered with millions of tiny fingerlike projections called villi. This photomicrograph shows one villus and its covering of epithelial cells, which absorb nutrients from digested food in the intestine.

Papain is an enzyme similar to the enzymes in your digestive tract that hydrolyze protein. It is used in some brands of meat tenderizer. (Some meat tenderizers also contain monosodium glutamate [MSG]. This material is not the active ingredient. It is added chiefly to improve flavor.) You can demonstrate the ability of the enzyme in meat tenderizer to hydrolyze protein by a simple

miniexperiment with gelatin. Gelatin is a protein that is extracted from connective tissues such as tendons and ligaments. You are most familiar with it under the trade name Jell-O.

B-31 Enzymatic Digestion of Protein

miniexperiment



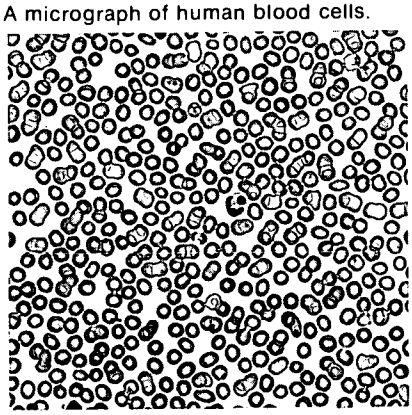
Add about 5 cm^3 of a 10-percent gelatin solution to a 50-cm^3 beaker. Set the beaker in an ice bath and allow the gelatin to solidify. Test the consistency of the gelatin by gently poking it with a glass rod.

When the gelatin has solidified, remove the beaker from the ice bath. Generously sprinkle some meat tenderizer over the gelatin and allow it to sit at room temperature. After 2 minutes, test the consistency of the gelatin by poking it gently with a glass rod. Repeat this test at 1-minute intervals. Pineapples contain an enzyme similar to the enzyme in meat tenderizer. Do you think you could make a Jell-O salad with fresh pineapple?

Do you think you could make a Jell-O salad with cooked or canned pineapple? Why?

The final products of all the digestive reactions are simple molecules. This is important because the cells of the intestine are selective. They will allow only certain molecules to pass into the blood. For example, sucrose, the common table sugar you use on your cereal, is a disaccharide. It cannot pass through the intestine into the blood. It must first be hydrolyzed to the monosaccharides, glucose and fructose. The same is true of lactose. Lactose is the disaccharide in milk. It cannot be absorbed into the blood unless it is hydrolyzed into the monosaccharides glucose and galactose.

A micrograph of human blood cells.



CAROLINA BIOLOGICAL SUPPLY COMPANY

B-32 The Components of Metabolism

After the digested compounds have passed through the wall of the intestine, they are carried by the blood to the tissues where they enter the cells. Inside the cells, the compounds undergo additional metabolic reactions.

What kinds of reactions take place inside cells? There are so many of them that they are almost innumerable. However, the reactions do not take place at random. Metabolism inside cells is a highly organized activity.

The oxidation of glucose to carbon dioxide and water provides a good example of the metabolism that occurs inside cells. We can write the equation for glucose metabolism as follows:

$$C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6\ CO_2 + 6\ H_2O + \text{energy}$$

This is the correct balanced equation for the overall metabolism of glucose. But the equation is deceptively simple. It leads you to believe that oxygen reacts directly with glucose in the body. This is simply not true. Oxygen reacts with other compounds that are produced at the end of a long series of reactions.

What is this series of reactions? Take a look at the following chart, *Three Pathways of Glucose Metabolism*. It shows an outline of the metabolism of glucose. Each arrow represents an enzymatic reaction that catalyzes a different step of the metabolism of glucose. Thus, you can see that glucose metabolism is a *stepwise process*. This is true of all metabolism. It proceeds by a series of reactions that occur in a *specific* order.

Any series of reactions in a cell is called a *metabolic pathway*. Actually there are three metabolic pathways shown on the following chart. Pathway I is called *glycolysis*. Pathway II is called the *Krebs cycle*. And Pathway III is called the *respiratory chain*. We will discuss these three pathways in this order so that you may understand how stored energy in glucose is released to produce the energy for the body's metabolism.

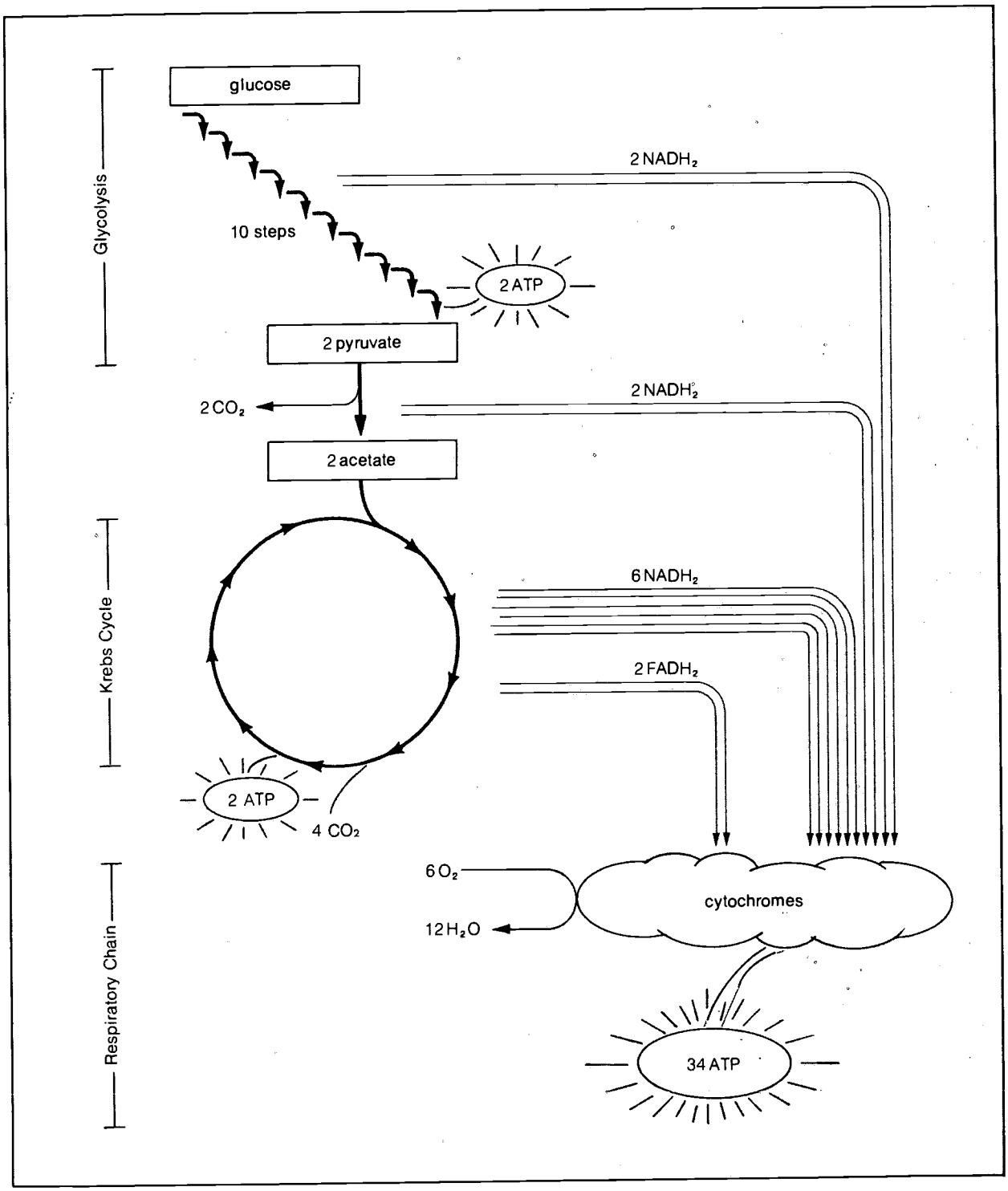
Each step in every pathway is catalyzed by a different enzyme. This is why cells must produce thousands of enzymes. Each enzyme reaction makes a small change in a molecule, and each change requires another enzyme. Each compound produced in the pathway is called a *metabolite*. The first step in glycolysis provides an example of a metabolic reaction. This reaction is shown in the following equation:

$$\text{glucose} + \text{ATP} \xrightarrow[\text{enzyme}]{\text{hexokinase}} \underbrace{\boxed{\text{glucose}-6-\text{phosphate}}}_{\text{metabolite}} + \text{ADP}$$

Glucose-6-phosphate is the metabolite produced by this reaction. Hexokinase is the enzyme that catalyzes the reaction. But what about ATP and ADP? Just as ATP stands for adenine *tri*phosphate, ADP stands for adenine *di*phosphate. They are not called *metabolites*. They belong to a class of compounds called *cofactors*.

Cofactors are substances that are required by enzymes in order to carry out their reactions. As a result, cofactors are an essential part of a metabolic pathway. If they are not present, the enzymes of the pathway will not be able to catalyze the reactions and

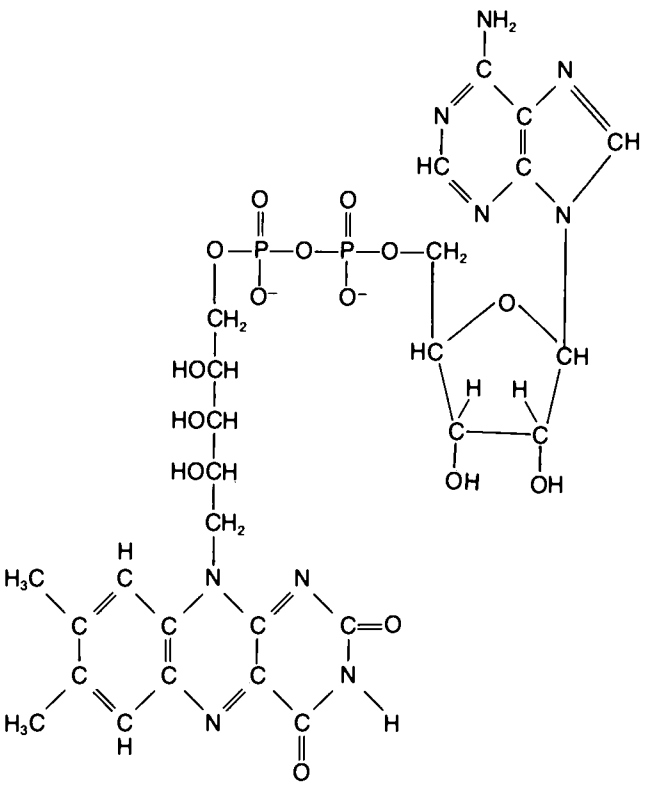
THREE PATHWAYS OF GLUCOSE METABOLISM



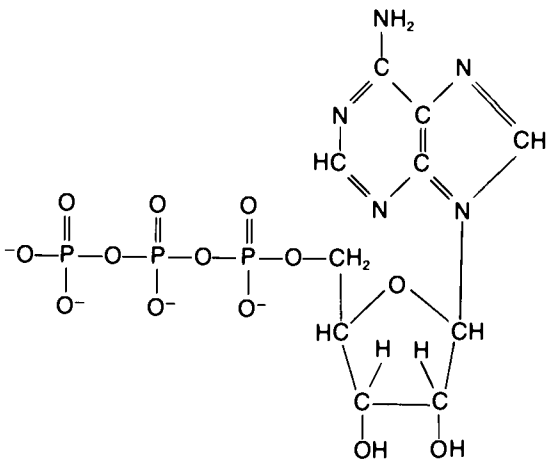
Outline of the three pathways that metabolize glucose to produce metabolic energy. Each arrow represents an enzyme reaction. Compounds produced by glycolysis and the Krebs cycle are utilized in the respiratory chain.

metabolism will not proceed. There are other cofactors in glucose metabolism. Two important ones are NAD and FAD. Their structures are shown in the following diagrams. They are obviously complex molecules. Their complex structure tends to obscure their biochemical reactions, so biochemists often use their abbreviations when they write reactions.

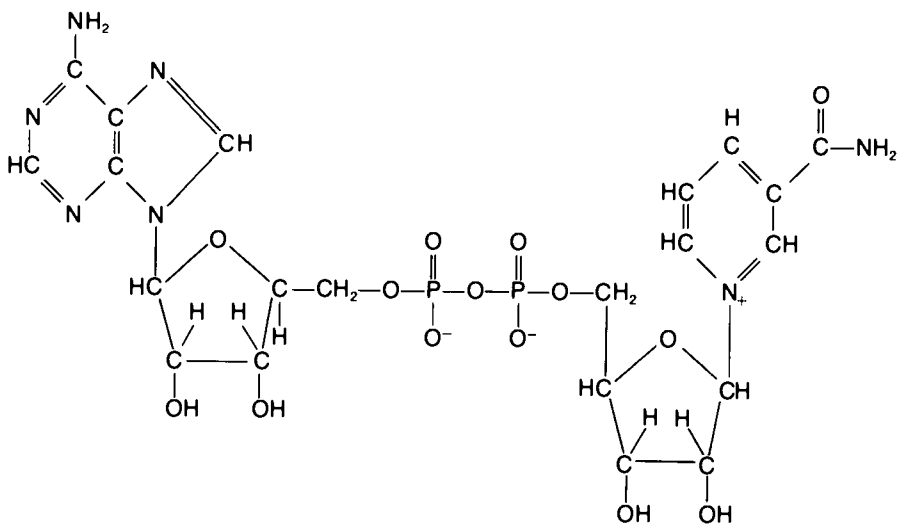
FAD



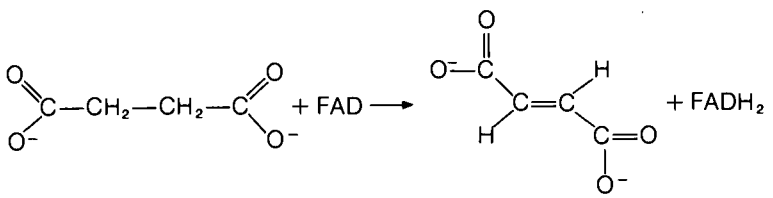
ATP



NAD



There are many different cofactors involved in metabolism, and they act in many different ways. However, the metabolic functions of NAD and FAD are similar. We will discuss FAD first. FAD is the cofactor that reacts with succinate dehydrogenase in the cell. (We used DPIP in the succinate dehydrogenase experiment just to make the reaction easy to measure. DPIP does not normally exist in the cell.) The succinate dehydrogenase reaction is shown in the following equation:



succinate fumarate

The two hydrogens are transferred from the succinate molecule to FAD to form $FADH_2$. The $FADH_2$, in turn, loses these two hydrogens in the respiratory chain and FAD is regenerated.

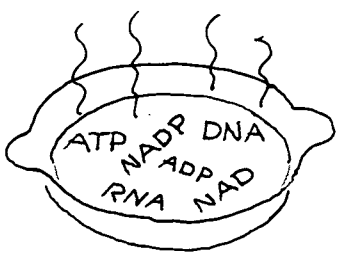
$$FADH_2 + \frac{1}{2} O_2 \xrightarrow[\text{chain}]{\text{respiratory}} H_2O + FAD$$

Thus, you can see that FAD acts as a shuttle to carry hydrogens back and forth between metabolic reactions.

NAD acts in much the same way as FAD. The function of NAD is to transfer hydrogen atoms from one reaction to another as shown here.

$$XH_2 + NAD \xrightarrow[1]{\text{enzyme reaction}} X + NADH_2$$

$$Y + NADH_2 \xrightarrow[2]{\text{enzyme reaction}} YH_2 + NAD$$



BIOCHEMICAL ALPHABET SOUP

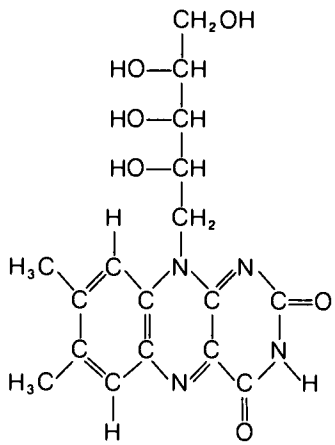
We have used X and Y to simplify the reactions. X and Y represent metabolites, such as pyruvate. There are actually many different compounds in metabolism that react like X and Y in these reactions. In the first enzyme reaction above, NAD is converted to $NADH_2$; and in the second enzyme reaction, $NADH_2$ is converted back to the original NAD. Thus, NAD simply serves as a shuttle for hydrogen atoms. It carries the hydrogen atoms from one enzyme reaction to another.

TIME MACHINE

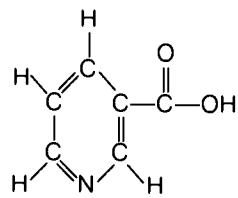
1903	A motorist makes the first auto trip across the United States (from San Francisco to New York).
1904	James M. Barrie's play *Peter Pan* opens in London.
1905	Albert Einstein theorizes that mass and energy are equivalent.
1905	**Scientists Harden and Young isolate the first cofactor, which is later shown to be NAD.**
1906	San Francisco is razed by fire and earthquake.
1907	James M. Spangler invents first electric vacuum cleaner.
1908	Jack Johnson is first black to become world heavyweight boxing champion.
1909	The National Association for the Advancement of Colored People (NAACP) is formed.

In addition to acting as hydrogen shuttles in metabolism, NAD and FAD have something else in common. Both molecules contain vitamins as part of their structures. A vitamin is an organic molecule which is required in small amounts by an organism but cannot be synthesized by the organism and therefore must be obtained in the diet. The vitamin riboflavin is used by your cells as a starting material for the synthesis of FAD. Similarly, the vitamin niacin is used for the synthesis of NAD.

RIBOFLAVIN



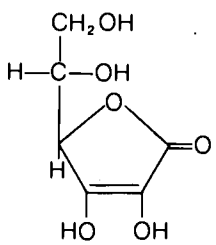
NIACIN



If you do not get enough of the vitamins niacin or riboflavin in your diet, your cells will not be able to synthesize all the NAD and FAD they need. Metabolism cannot proceed without these cofactors, and serious illnesses may result if a vitamin deficiency is severe enough. A deficiency in niacin results in the disease called *pellagra*. This disease is common in impoverished parts of the world where there is famine, and it results in thousands of deaths annually.

Many other diseases are caused by vitamin deficiencies. A deficiency in vitamin C (also a cofactor in metabolism) causes the disease called *scurvy*. There is an interesting story about the discovery of vitamin C. When it was isolated by Albert Szent-Györgyi in 1924, its exact structure was unknown. Because it was a carbohydrate, he felt the name should be given the ending *ose* like glucose and other carbohydrates. Since he was *ign*orant of the exact structure, he suggested the name *ign*ose. This was too undignified for some biochemists; so Szent-Györgi replied, "Why not call it *Godnose?*" Later he named it ascorbic acid. Despite his humor, he received the Nobel Prize for his work.

ASCORBIC ACID
(Vitamin C)



B-33 Glycolysis: A Metabolic Pathway

In the previous section we mentioned that there are three pathways involved in the complete metabolism of glucose. The first pathway, called *glycolysis*, is shown in the following figure. You can see that there are ten enzymatic steps in this pathway. At each step, an enzyme makes a relatively small change in the structure of a metabolite. Four of the steps involve ATP and two steps involve the cofactor NAD. The overall metabolism of glucose by the entire glycolysis pathway can be summarized by the following reaction:

GLUCOSE

$$C_6H_{12}O_6 + 2\,NAD + 2\,ADP + 2\,H_2PO_4^- \longrightarrow$$

phosphate

$$2\,C_3H_3O_3^- + 2\,NADH_2 + 2\,ATP + 2\,H_2O$$

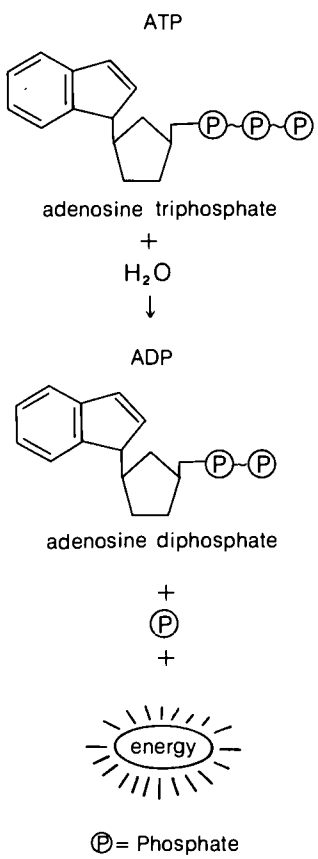
pyruvate

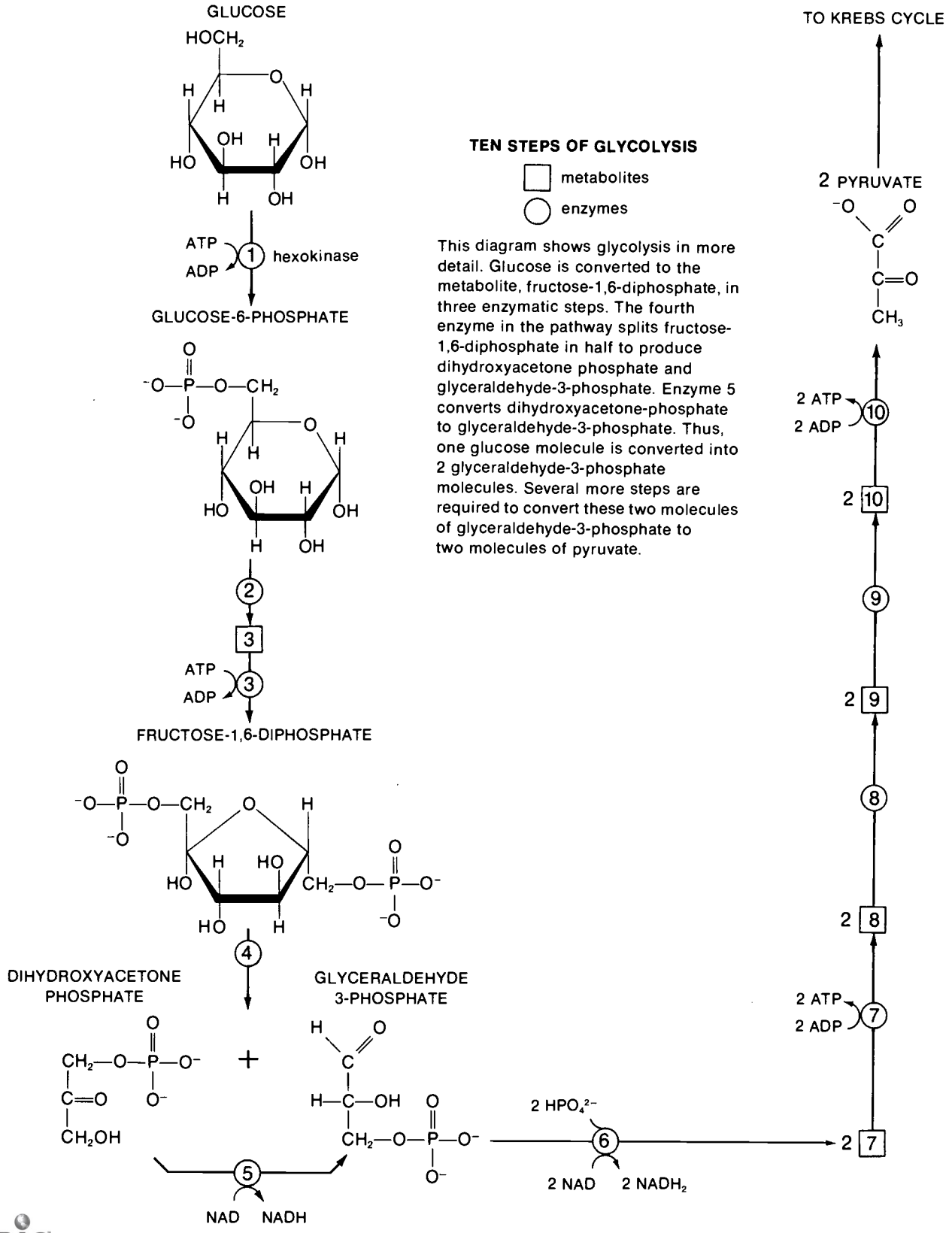
Thus, during glycolysis the six-carbon glucose molecule is split in half and converted to two three-carbon pyruvate molecules. Notice that this pathway does not produce CO_2 and does not involve oxygen from the air. However, two molecules of $NADH_2$ and two molecules of ATP are produced for every glucose that enters the pathway. The $NADH_2$ is a hydrogen shuttle that will carry hydrogen to other metabolic reactions. (We will say more about this later.)

But what about ATP? You have probably heard that metabolic energy is stored in ATP. This is entirely correct. But you might wonder how ATP stores energy. The energy stored in the ATP molecule is released when ATP is hydrolyzed. During the hydrolysis of ATP, one of the phosphate groups which forms the tail of the molecule is removed.

The energy released when ATP is hydrolyzed is used to run the energy-requiring reactions of your body. For example, you use the energy released by the hydrolysis of ATP to contract your muscles. Many metabolic reactions also require energy. It takes energy to synthesize macromolecules such as proteins. It takes energy to synthesize nucleic acids such as DNA. It also takes energy to synthesize lipids and polysaccharides. Most of this energy comes from the hydrolysis of ATP.

The reaction of $NADH_2$ and oxygen is connected to another reaction in the respiratory chain. Every time an $NADH_2$ molecule reacts at the respiratory chain, three ATP molecules are synthesized. Consequently, all of the $NADH_2$ produced by glucose metabolism can be used for the formation of ATP.



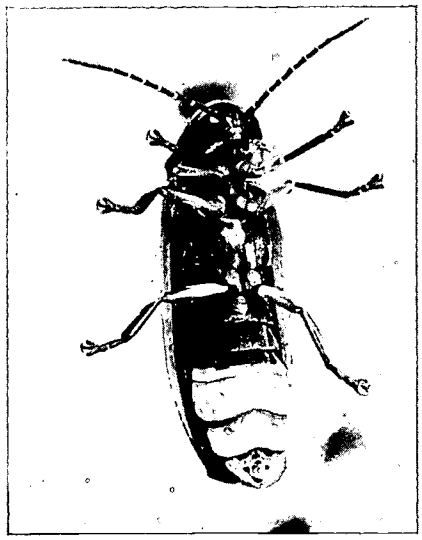

GLUCOSE
ATP
ADP
1
hexokinase
GLUCOSE-6-PHOSPHATE
2
3
ATP
ADP
3
FRUCTOSE-1,6-DIPHOSPHATE
4
DIHYDROXYACETONE PHOSPHATE
GLYCERALDEHYDE 3-PHOSPHATE
5
NAD
NADH
2 HPO4 2-
6
2 NAD
2 NADH2
2 7
2 ATP
2 ADP
7
2 8
8
2 9
9
2 10
2 ATP
2 ADP
10
2 PYRUVATE
TO KREBS CYCLE
TEN STEPS OF GLYCOLYSIS
metabolites
enzymes
This diagram shows glycolysis in more detail. Glucose is converted to the metabolite, fructose-1,6-diphosphate, in three enzymatic steps. The fourth enzyme in the pathway splits fructose-1,6-diphosphate in half to produce dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. Enzyme 5 converts dihydroxyacetone-phosphate to glyceraldehyde-3-phosphate. Thus, one glucose molecule is converted into 2 glyceraldehyde-3-phosphate molecules. Several more steps are required to convert these two molecules of glyceraldehyde-3-phosphate to two molecules of pyruvate.

Thus, in a sense, ATP acts as a shuttle, much as NAD and FAD do. ATP picks up the energy produced by one metabolic pathway (such as glycolysis) and then shuttles the energy to other metabolic reactions where it is needed.

We are unable to see directly most of the reactions that utilize ATP, but there is one shining example of a visible reaction that uses ATP. Fireflies use their lanterns to attract a mate. The lanterns contain a chemical called *luciferin* and an enzyme called *luciferase*. In the presence of magnesium ions (Mg^{2+}), the luciferase catalyzes a reaction between luciferin, oxygen, and ATP. In the process ATP is hydrolyzed, and light is given off. Let's look at this reaction.

B-34 Making Light With ATP

miniexperiment



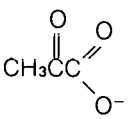
Put 10 to 12 firefly lanterns into a mortar. Add 1 g sand (this need not be precisely measured—a pinch will do). Then add 2 to 3 cm^3 of pH 7.3 phosphate buffer. Grind the lanterns, sand, and buffer for at least 5 minutes. The sand serves as an abrasive to break cells, and the buffer extracts the desired chemicals.

Once the enzyme is extracted (the liquid should be very cloudy), carefully pour off (decant) the liquid from the mortar into a clean test tube. Add 10 to 20 crystals of magnesium sulfate ($MgSO_4 \cdot 7H_2O$). Magnesium is required for the reaction. Mix well.

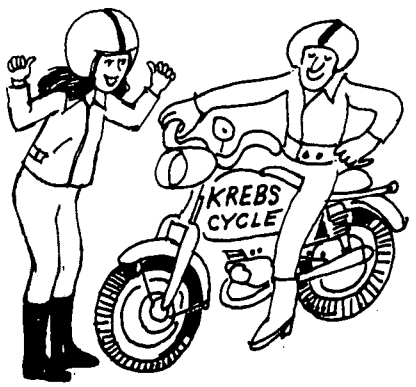
Obtain 2 to 3 drops of ATP solution. Take the test tube of enzyme into a dark area. When your eyes have become accustomed to the dark, add the ATP to your enzyme solution. What do you see?

The light given off by the solution is a result of the reaction catalyzed by the enzyme from the firefly lanterns. The energy required for this reaction is supplied by the hydrolysis of ATP. In a similar way ATP provides the energy needed in many of the reactions in our own bodies. In addition to the reactions of metabolism mentioned before, ATP is necessary for virtually all of our everyday activities—talking, walking, thinking, seeing. It should be obvious that a great deal of ATP is required. Where does all of this ATP come from? During glycolysis, as we have seen, the metabolism of each molecule of glucose provides only two molecules of ATP. Much more ATP is produced as a result of the further metabolism of pyruvate, the product of glycolysis.

PYRUVATE



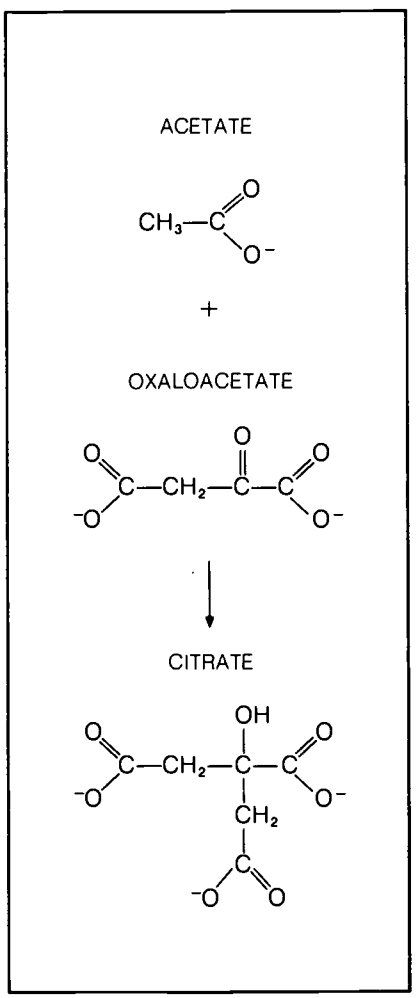
B-35 The Krebs Cycle

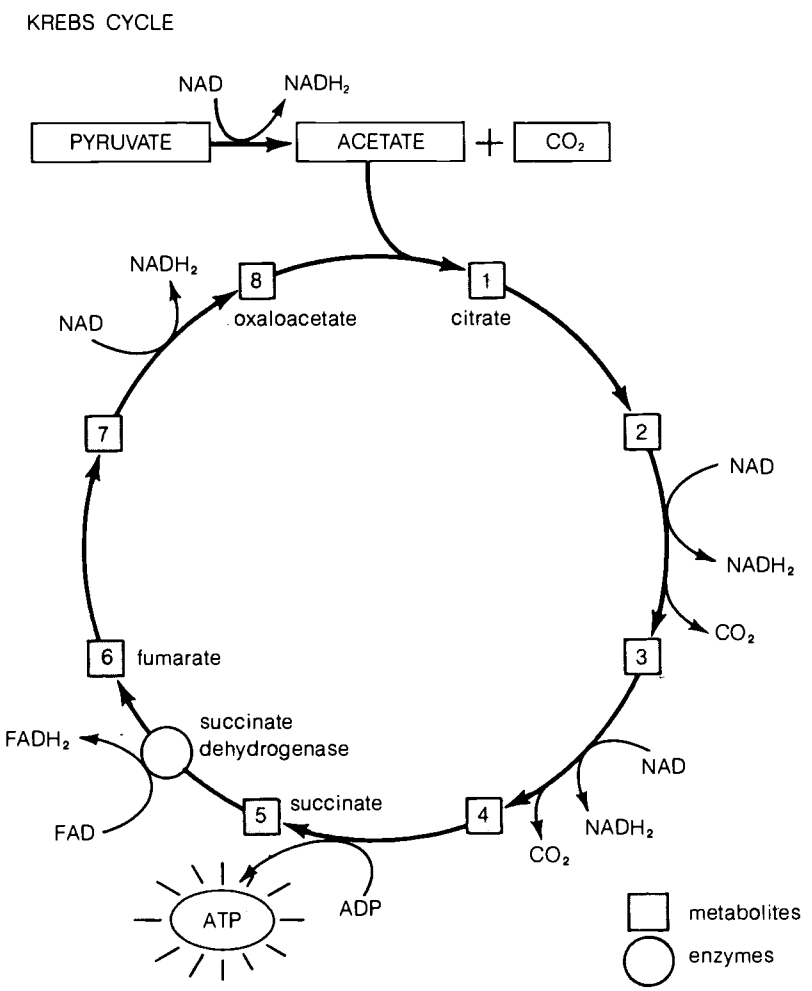


You now know that each molecule of glucose that enters glycolysis is converted into two molecules of pyruvate. The pyruvate can be broken down further to provide more metabolic energy. This process involves the last two pathways of glucose metabolism: the Krebs cycle and the respiratory chain.

The pyruvate produced by glycolysis is first converted into acetate. The acetate can then enter the Krebs cycle. An outline of the Krebs cycle is given in the following diagram. Every molecule of pyruvate that enters this pathway is broken down completely into *three* molecules of carbon dioxide. As this is done, one molecule of ATP, one molecule of $FADH_2$, and four molecules of $NADH_2$ are produced.

In the Krebs cycle, the acetate (a two-carbon molecule) combines with oxaloacetate (a four-carbon molecule) to produce citrate (a





six-carbon molecule). As the citrate is metabolized in the Krebs cycle, two carbon atoms are lost as carbon dioxide (CO_2), and the four-carbon molecule oxaloacetate is regenerated. Thus, you can see why this pathway is called a cycle. Oxaloacetate is both a starting material and a product in this pathway. The cycle will continue as long as acetate is supplied. In fact acetate is the link between glycolysis and the Krebs cycle.

For every molecule of glucose that enters glycolysis, two molecules of pyruvate are produced. These molecules of pyruvate are broken down completely to six molecules of carbon dioxide. As this is done, two molecules of ATP, two molecules of $FADH_2$, and four molecules of $NADH_2$ are produced.

We started with glucose at the beginning of glycolysis and obtained carbon dioxide during the Krebs cycle. But we still have not used the oxygen from the air, which, as you know, is also involved in glucose metabolism. This is where the cofactors $NADH_2$ and $FADH_2$ play an important role.

In animals (including human beings), most of the $NADH_2$ produced by glucose metabolism is used up by the *respiratory chain*.

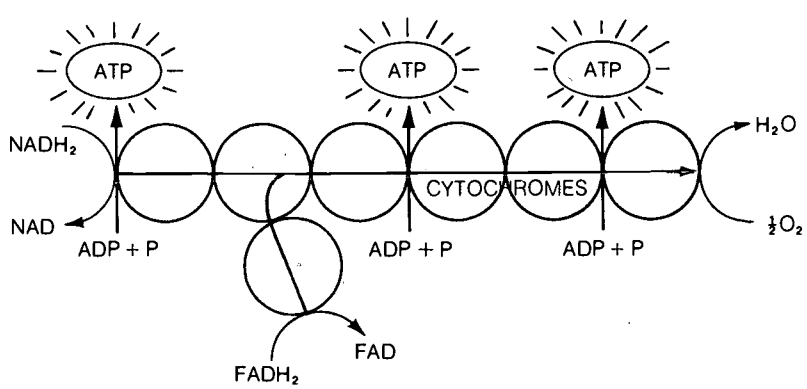
TIME MACHINE

1935	Carl C. Magee invents the parking meter.
1936	Jesse Owens wins four gold medals for track in Berlin Olympics.
1937	Joe Louis gains heavyweight boxing title, which he retains for 11 years.
1937	**Hans Krebs proposes the citric acid cycle.**
1938	Teflon is accidentally discovered by a Du Pont chemist.
1939	Igor Sikorsky's first successful helicopter takes to the air.
1940	Franklin Delano Roosevelt is elected to a third term.

B-36 The Respiratory Chain

The respiratory chain is made up of a large number of enzymes. Some of these enzymes contain the *heme* group, which is also found in catalase and the hemoglobin of the blood. The heme group is responsible for the deep red color of hemoglobin. The heme-containing enzymes of the respiratory chain are also deeply colored. These enzymes are called *cytochromes* (cyto = cell; chrome = color). The heme group is capable of reacting with oxygen.

RESPIRATORY CHAIN



Each circle represents an enzyme in the respiratory chain. $NADH_2$ and $FADH_2$ react with different respiratory chain enzymes. The reaction of each $NADH_2$ molecule sets up a chain of events that leads to the synthesis of three ATPs. However, only two ATPs are produced for each $FADH_2$ reaction that occurs.

The respiratory chain catalyzes the reaction between $NADH_2$ or $FADH_2$ and oxygen from the air. This is the reaction in glucose metabolism that directly involves oxygen from the air. Thus, you can see that oxygen does not react directly with the glucose molecule or any of its metabolites. It reacts with the respiratory chain. In this chain, oxygen is converted to water, $NADH_2$ is converted back to NAD, and $FADH_2$ is converted to FAD.

Thus, the function of $NADH_2$ in glucose metabolism is to carry hydrogen atoms from the enzyme-catalyzed reactions of glycolysis and the Krebs cycle to the respiratory chain. The respiratory chain catalyzes reactions that convert the hydrogens carried by the $NADH_2$ to water.

However, there is more to the story. One of the most remarkable things about the respiratory chain is the large amount of ATP it produces. Every time an $NADH_2$ molecule reacts at the respiratory chain, three ATP molecules are synthesized. In a similar way two ATP molecules are synthesized whenever $FADH_2$ reacts at the chain. Consequently, all of the $NADH_2$ and $FADH_2$ produced by glucose metabolism can be used for the formation of ATP (see the preceding diagram: *Respiratory Chain*).

The overall reaction for the complete metabolism of glucose is

$$C_6H_{12}O_6 + 6\ O_2 + 38\ ADP + 38\ phosphate \longrightarrow 6\ CO_2 + 38\ ATP + 44\ H_2O$$

Most of the ATP produced by the complete metabolism of glucose comes from the reactions of $NADH_2$ and $FADH_2$ at the respiratory chain. Relatively little ATP is supplied by glycolysis. This is summarized in Table 4. This helps explain why oxygen is so important in the metabolism of so many organisms. The oxygen is a reactant in the respiratory chain that provides such a large amount of ATP.

TABLE 4: BALANCE SHEET OF COFACTORS IN GLUCOSE METABOLISM

	Moles of Cofactor per Mole Glucose Consumed		
PATHWAY	**$NADH_2$**	**$FADH_2$**	**ATP**
Glycolysis	2 produced	0	2 produced
Krebs cycle	8 produced	2 produced	2 produced
Respiratory chain	10 consumed	2 consumed	34 produced
Total produced	0	0	38

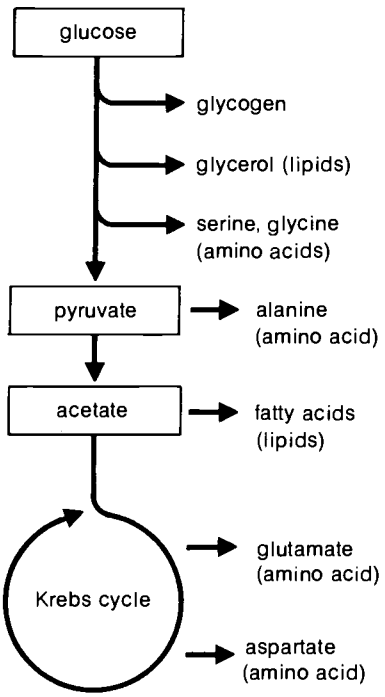
B-37 Branching

Glucose metabolism is an important source of ATP. However, you should not get the idea that this is the only function that glucose has in metabolism. In fact the metabolites produced during glucose glucose metabolism can be used as starting materials for the synthesis of other compounds such as triglycerides (and other lipids) and some amino acids. Also, as we have mentioned before, excess glucose is converted to the polysaccharide glycogen, which is stored until glucose is needed. This is possible because the glycolysis pathway contains *branch points* at which other pathways begin. The same is true of the Krebs cycle. Some of the metabolites produced by the Krebs cycle can be used by other pathways for the production of different compounds, such as amino acids.

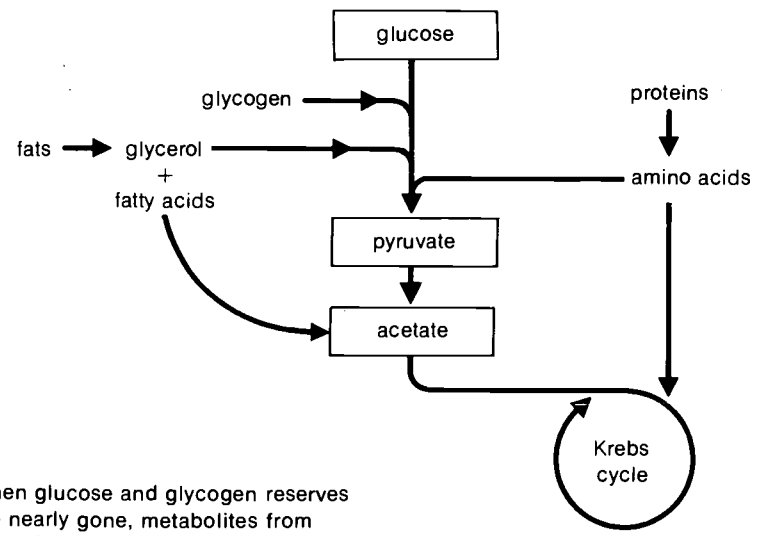
Furthermore, glucose is not the only molecule that is metabolized to produce metabolic energy. Proteins and fats (triglycerides) can also be broken down to produce ATP. Fats are used most rapidly when the carbohydrates such as glucose and glycogen have been consumed. This is what happens when a person goes on a diet. As the glucose and glycogen are used up, the body begins to utilize fats that have been stored as energy reserves. The molecules of fat are broken down to fatty acids and glycerol. The glycerol can enter glycolysis and be used to produce ATP. The fatty acids cannot enter glycolysis. They are broken down to acetate. The acetate can then enter the Krebs cycle (see diagram, *Entrance of Fats, Proteins, and Amino Acids into Metabolism*) and be used to produce ATP.

BRANCHING

The metabolites of glycolysis and the Krebs cycle can be used for the synthesis of other compounds. Some of the major branch points in these pathways are shown here.



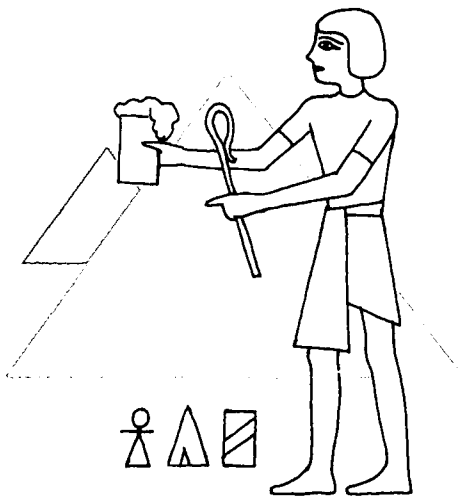
ENTRANCE OF FATS, PROTEINS, AND AMINO ACIDS INTO METABOLISM



When glucose and glycogen reserves are nearly gone, metabolites from fat and, eventually, from protein metabolism enter into glycolysis to be used for metabolic energy.

We also begin to use proteins for energy when carbohydrate reserves become depleted. To do this, the proteins are broken down to amino acids. Many of these enter glycolysis and the Krebs cycle, as illustrated in the preceding diagram, *Entrance of Fats, Proteins, and Amino Acids into Metabolism.*

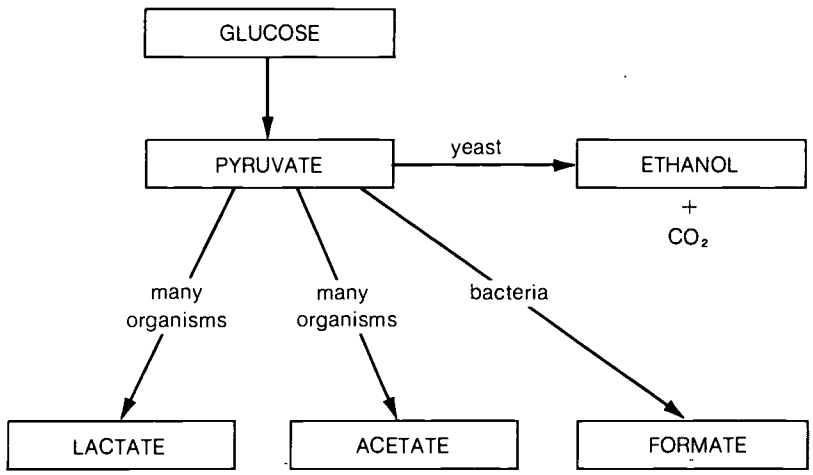
So far we have been considering metabolic pathways in animals. However, metabolic pathways are not completely identical in all organisms. We will have more to say about this in the next section.



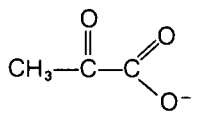
B-38 The Versatility of Metabolism

In what ways are vinegar, yogurt, beer, and penicillin the same? They are all the result of fermentation. But what is fermentation? Fermentation is the process by which such diverse products as yogurt, sauerkraut, wine, vitamins, and antibiotic medicines are produced by the metabolism of microorganisms.

Ancient peoples knew the art of fermentation. The Egyptians left hieroglyphics that describe the making of beer, and Noah knew how to make wine. For thousands of years people did not understand what occurred during fermentation. Now we can explain some fermentations in terms of metabolism. Although microorganisms can metabolize glucose to pyruvic acid, the pyruvate does not always enter the Krebs cycle. Instead, it is converted to other products as illustrated in the following diagram.



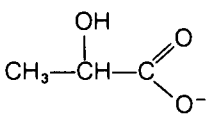
PYRUVATE



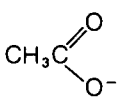
ETHANOL

CH_3CH_2OH

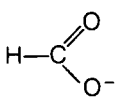
LACTATE



ACETATE



FORMATE



As you know, yeast produces ethanol (ethyl alcohol). Ethanol is a product of pyruvic acid metabolism. This fermentation is fundamental to the manufacture of all kinds of alcoholic beverages and is also important in baking. Bread dough rises as a result of the carbon dioxide gas that is produced when ethanol is formed

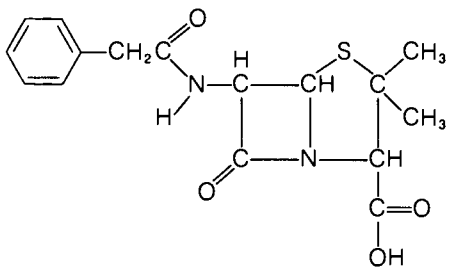
Most breadmaking today is based on yeast fermentation, which usually requires several stages of rising at 29°C or more. In large-scale commercial baking, however, fermentation takes place in a liquid that is added to the flour and fed in gigantic batches through a continuous system that accelerates the entire process.

from pyruvic acid. Other organisms produce other compounds from pyruvic acid. Yogurt, buttermilk, sour cream, and some cheeses are products resulting from fermentation of dairy products by lactic acid producing bacteria. Vinegar is a weak acetic acid solution produced by certain bacteria.

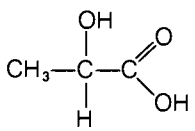
Even human beings can make lactic acid from pyruvic acid. This occurs in muscles during heavy exercise. Under these conditions the muscles use up the available oxygen, leaving the muscle cells without enough oxygen to run the respiratory chain. When this happens, the pyruvic acid does not enter the Krebs cycle. Instead, it is converted to lactic acid, which is then released into the blood stream. Later the lactic acid is converted back to glucose by the liver.

Not all compounds produced in fermentation come from pyruvic acid or glucose metabolism. Examples of these include several vitamins such as niacin, riboflavin, and vitamin C. In addition, fermentations are used in the production of many steroid drugs and antibiotics. You are probably familiar with the antibiotic penicillin. You can see from its structure that penicillin cannot be produced directly from pyruvic acid. It is produced by the mold *Penicillium chrysogenum*. Another member of the *Penicillium*

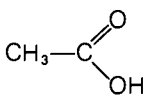
PENICILLIN

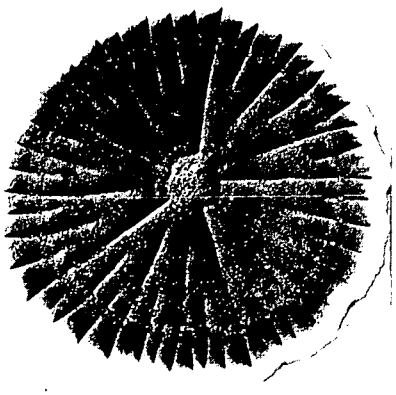


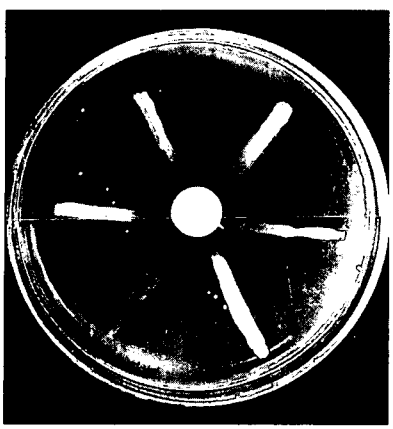
LACTIC ACID



ACETIC ACID



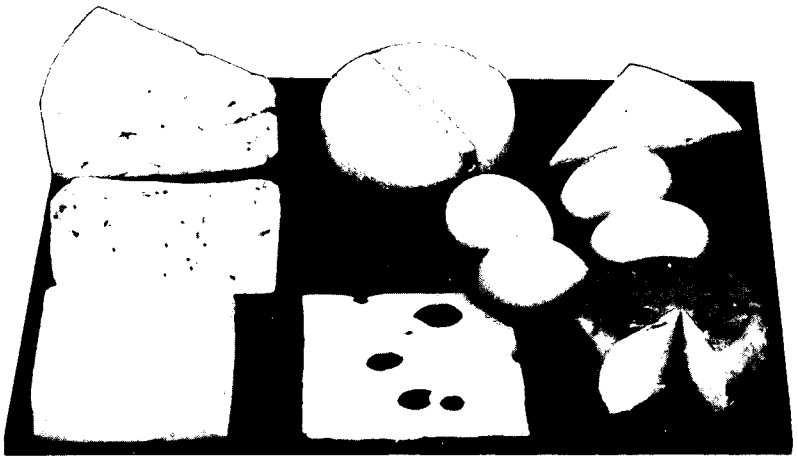




Mold, *Penicillium chrysogenum* (*left*), is used to produce the antibiotic we know as penicillin. In the process the mold is grown in large fermentation vessels. Antibiotics are continually being tested (*right*) to determine their effectiveness against disease-producing organisms.

Mold spore cases magnified 900 times.

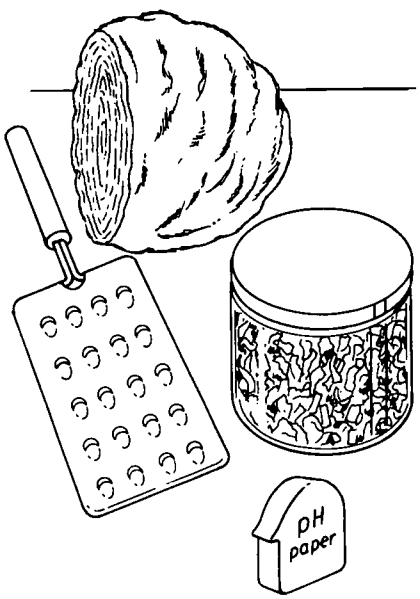
group of molds is used to produce blue cheeses (Roquefort, Gorgonzola). Thus, the metabolism of microorganisms is very useful to us.



Today most fermented products are produced on a large scale by industry, making it possible for you to buy most of them in the supermarket or drugstore. But for years people made many of these products at home. Sauerkraut is one fermented product that is particularly easy to make.

EXPERIMENT

B-39 Making Sauerkraut



You can prepare your sauerkraut in a 120-cm^3 wide-mouth bottle. Tightly pack shredded cabbage into the bottle until it is about one-fourth full. Sprinkle about 0.3 g of sodium chloride evenly over the cabbage. Pack in more cabbage until the bottle is about one-half full and add about the same amount of salt.

Repeat these steps two more times until the bottle is full. (The salt serves to draw the juice out of the cabbage to provide a good environment for the growth of the bacteria. However, you should be careful not to put in extra salt because too much salt can inhibit the growth of the bacteria.)

Add cold water to the bottle until the cabbage is completely submerged. Be sure that you pack the cabbage in tightly to remove as many trapped air bubbles as possible.

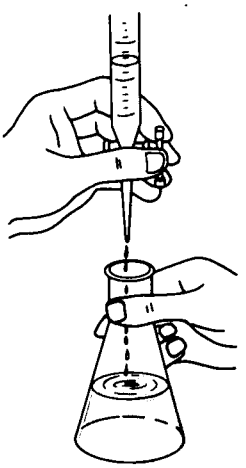
Test the pH of the solution with a piece of universal pH paper. Place the cap on the bottle. Do *not* screw it down tight. Leave it loose so that any gas generated by the fermentation can escape. Let your cabbage stand at room temperature for 10 to 14 days. Be sure the cabbage stays covered with water the whole time.

When the sauerkraut is ready (after 10 days), squeeze the juice out into a beaker. Determine the pH of the juice with a piece of universal pH paper.

Place 10 cm^3 of sauerkraut juice into an Erlenmeyer flask. Add 10 cm^3 of water and 2 drops of phenolphthalein indicator solution.

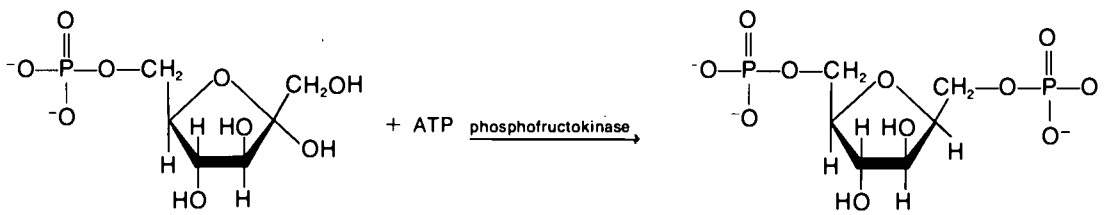
Titrate the sauerkraut juice with 0.05 M NaOH until it first turns "just pink." The pink color should remain when you gently swirl the flask. You will need to record the buret reading at the start of titration and the buret reading at the end point.

Calculate the number of moles of acid in 10 cm^3 of your sauerkraut juice.



EXERCISES

1. Triglycerides, proteins, and starch are important parts of the diet. They are different molecules, and their complete metabolism takes place through different metabolic pathways. However, they all undergo a similar kind of reaction as the first step in their metabolism. What do we call this kind of reaction? Where does it take place?
2. Name the three pathways of glucose metabolism.
3. For the following reaction identify (a) the enzyme, (b) a metabolite, and (c) a cofactor.

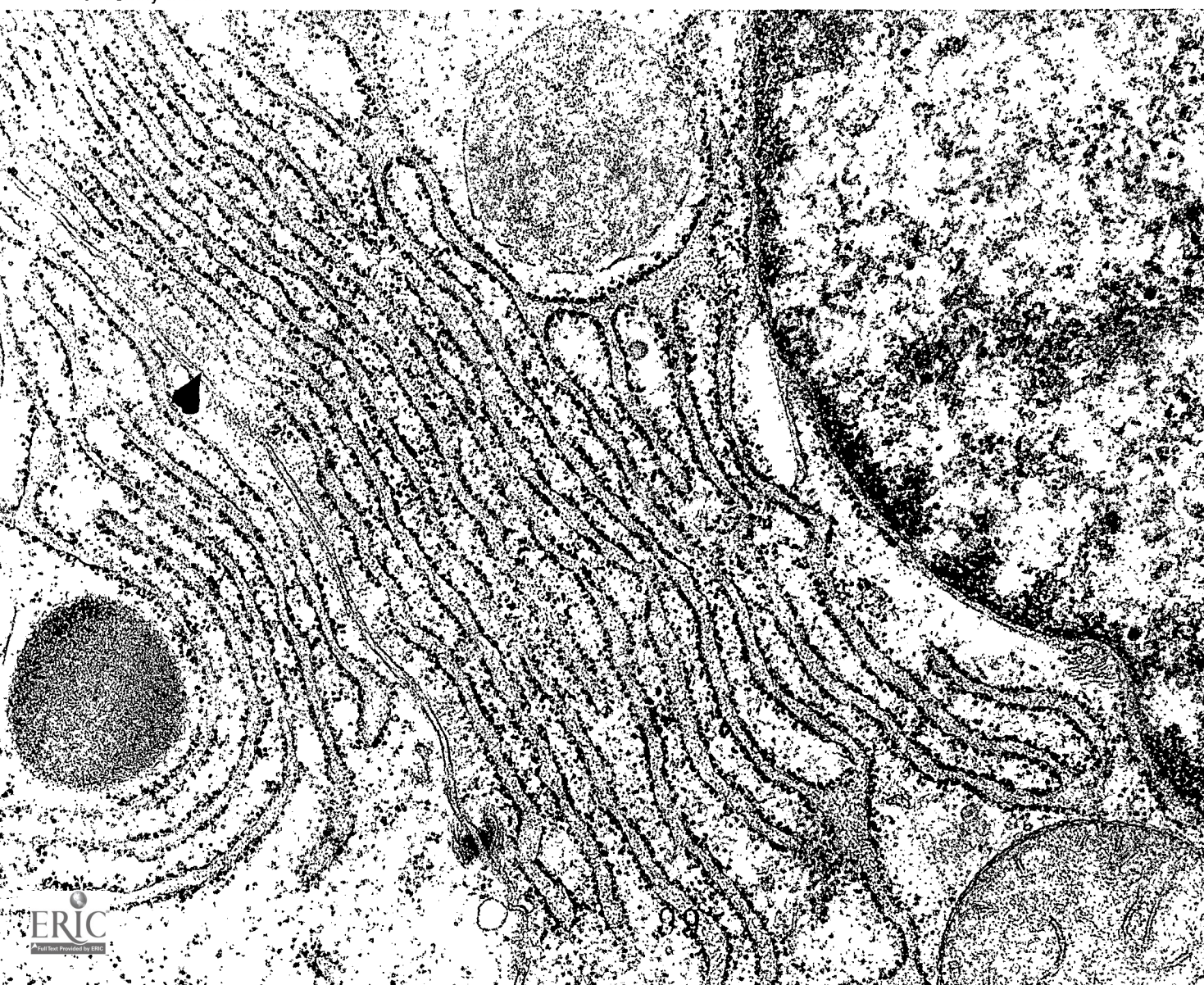


4. Considering the three pathways of glucose metabolism, which one
   (a) produces the most $NADH_2$?
   (b) produces the most ATP?
   (c) produces CO_2?
   (d) requires molecular oxygen as a reactant?
   (e) contains the enzyme succinate dehydrogenase?
   (f) converts six-carbon molecules to three-carbon molecules?
   (g) converts two-carbon molecules to carbon dioxide?
   (h) produces $FADH_2$?
   (i) converts $NADH_2$ to NAD?
5. How does yeast cause bread dough to rise?

The Organization of Cellular Activities

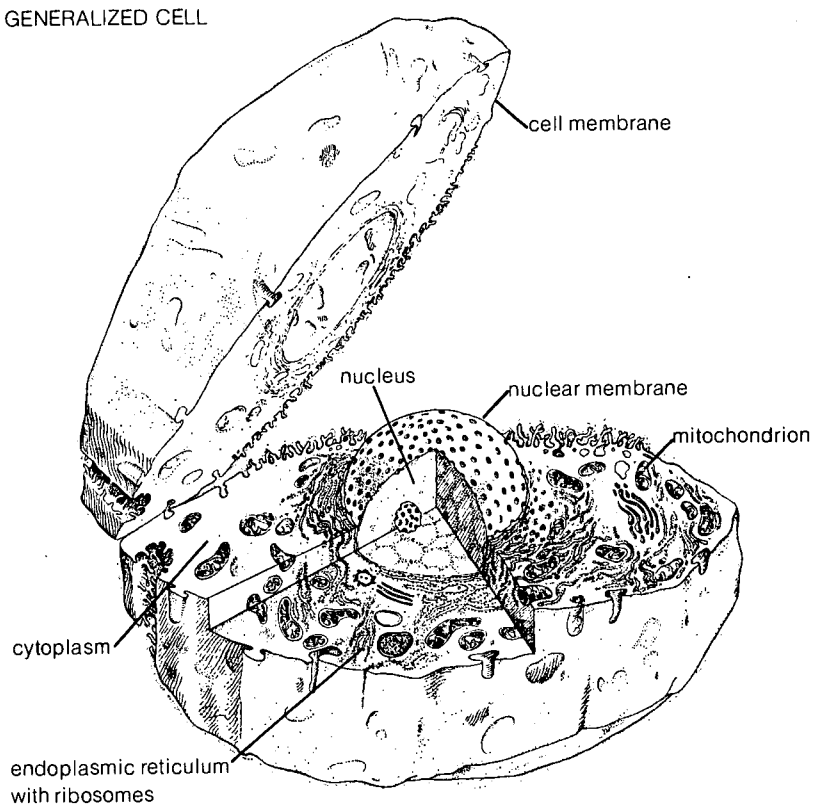
Biochemistry is more than just the study of the structures and metabolism of biomolecules. If we are to explain how these molecules function in living systems such as our own, we have to broaden our understanding by examining the biochemical organization within cells. What is a cell, what are its parts, and how do they function? How do cells reproduce and synthesize complex biomolecules? Let's look inside and see.

This electron micrograph shows several subcellular organelles found in animal cells. After you have read this section try to identify them.



B-40 Organelles: Little Organs in Cells

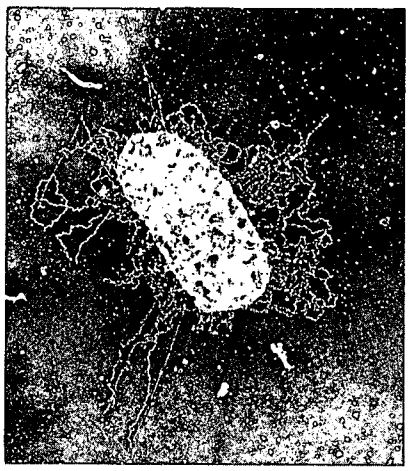
Early biochemists had a simple view of the cell as a bag filled with a mixture of enzymes, metabolites, and other molecules. We know better now. Studies using instruments such as the electron microscope have shown that each cell is elaborately subdivided into subcellular bodies such as the *nucleus* and the *mitochondrion*. These subcellular bodies are called *subcellular organelles*.



As you can see in the preceding diagram, each subcellular organelle has a different structure. As you might imagine, the different organelles have different functions. But how do they differ? Are certain metabolic pathways associated with specific subcellular organelles?

The answers to these questions were not easy to obtain. But now we know that some metabolic pathways are found in certain subcellular organelles. For example, the *Krebs cycle* enzymes and the enzymes associated with the *respiratory chain* are found in the mitochondria. Similarly, in plant cells all the *photosynthetic reactions* take place in an organelle called the *chloroplast*.

An electron micrograph of a disrupted bacterial cell reveals the complexity of the cell interior. The loops coming out from the cell are made of the bacterial DNA.

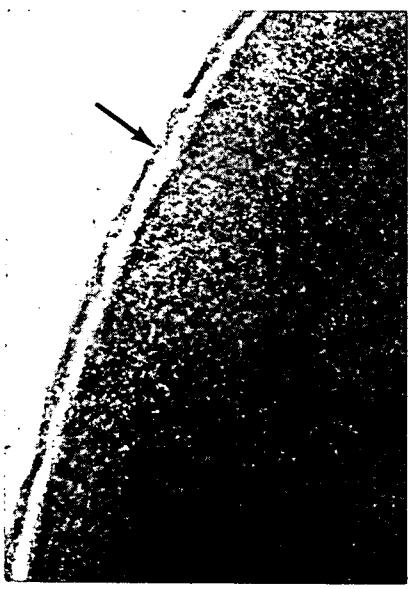


On the other hand, some metabolic pathways do not appear to be associated with any particular organelle. An example is glycolysis. The glycolytic enzymes are found in the *cytoplasm*. The cytoplasm is the fluid that fills the space between the subcellular structures. The glycolytic enzymes are dissolved in this fluid.

By and large, most enzymes are believed to be located within a particular organelle. Since each organelle possesses a different combination of enzymes, each functions in a different way. Thus, the subcellular organelles are actually portions of the cell that specialize in certain functions.

Although all cells have subcellular organelles, not all cell types have the same ones. For example, plant cells have chloroplasts, but animal cells do not. Both plant and animal cells have nuclei and mitochondria, but bacterial cells have neither. Even within an organism, cells may have different kinds and numbers of subcellular organelles. An extreme example is the red blood cell in mammals. Mature red blood cells have no subcellular organelles. Of course, they do have a cell membrane. Although there are many subcellular organelles, we will consider only a few of them.

B-41 Cell Membrane: Gateway to the Cell



The cell membrane (arrow) is vital to the survival of cells. It is about eight millionths of a millimeter thick (8 nanometers). The cell membrane controls the passage of molecules into and out of the cell, allowing nutrients to enter and wastes to leave, keeping essential molecules such as metabolites inside.

The membrane that separates the cell contents from the extracellular environment is called the *cell membrane*. This membrane has a structural role, as all membranes have. In addition, it transports nutrients into the cell and waste products out of the cell. The transport process is vital to the survival of the living cell.

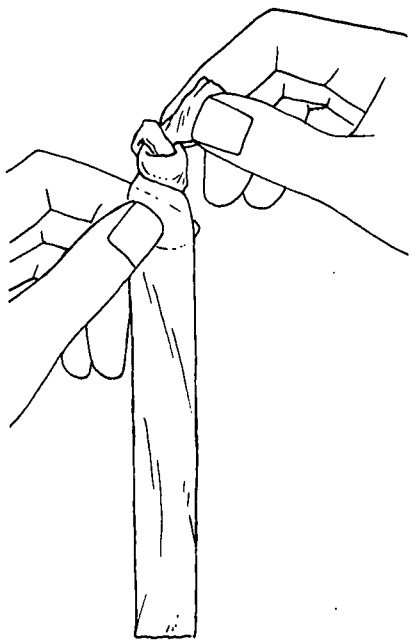
It is a well-known fact that certain molecules can pass through the membrane barrier and that other compounds are prohibited from passing into or out of the cell. A good example of the selective nature of the cell membrane is the fact that the cell contents are high in potassium ions and low in sodium ions. The extracellular environment is exactly the reverse. (It is high in sodium ions and low in potassium ions.) Many theories have been proposed to account for this universal finding. The theory that best explains all the known facts about the sodium and potassium content of cells has been called the *sodium-potassium pump* mechanism.

This theory proposes that cell membranes contain an enzymelike mechanism that literally pumps sodium ions out of the cell as fast as they enter the cell. This process requires energy. As you might expect, ATP is used by the cell to supply the energy needed. Many other biomolecules such as glucose and amino acids are also thought to be moved across cell membranes by mechanisms somewhat like the sodium-potassium pump. The exact details of these mechanisms are by no means completely understood.

There is another way that biomolecules can move across biological membranes. The molecules simply diffuse across. But even in this process only certain molecules can pass through cell membranes. Since biological membranes are difficult to work with, we will use a simple artificial membrane to study this process in the next experiment.

B-42 Artificial Membranes

EXPERIMENT



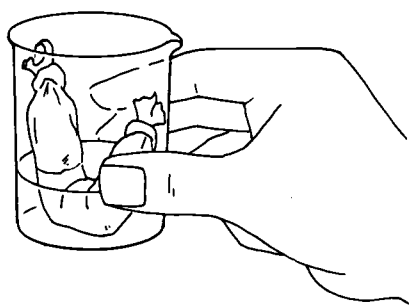
In this experiment you will use dialysis tubing as an artificial membrane. You will make a dialysis bag with the tubing and observe which biomolecules (starch, glucose, glutamate, and gelatin) diffuse through the membrane. Some of you will place starch and glucose in the bag and others will work with gelatin and glutamate.

Soften your piece of dialysis tubing by soaking it in water for a minute or two. When the tubing is soft, tie a knot in one end to form a bag.

Open the other end of the tubing by inserting the sharpened end of a pencil or the corner of a ruler. *Note:* Do not open the tubing by blowing into it. Why?

Fill the dialysis bag with 5–6 cm^3 each of glucose and starch or gelatin and glutamate solutions. Seal the bag by tying a knot in the open end of the bag. Rinse the bag *briefly* in water to wash away any spillage from the outside. Then place the bag in a small beaker containing about 10 cm^3 of water and let it soak for at least 30 minutes. (Carefully swirl the beaker periodically to mix the contents.)

After the dialysis bag has soaked for about 30 minutes, test the fluid outside the bag. What tests will you perform? (See section *B-13*.)



Questions:

1. You found that only one of the two types of molecules passed through the dialysis membrane. How can you account for this?
2. If the cell membranes worked by similar principles, which of the following biomolecules would remain within the cell?

succinate dehydrogenase	ethanol (ethyl alcohol)
serine	ascorbic acid
fructose	catalase

3. Of course, the dialysis tubing does not behave exactly like a biological membrane. Why?

Normal kidneys act as filters that remove wastes and poisons from the blood. Nowadays, a person whose kidneys are diseased or failing may be "hooked up" to an artificial kidney machine two or three times a week to undergo blood-cleaning by dialysis.

B-43 Artificial Kidney Machine

A membrane similar to dialysis tubing is used in the artificial kidney machine. The primary function of the kidney is to remove by-products of metabolism. Many of these by-products are small molecules that are poisonous in high concentrations. Normally, these are removed by the kidney as it forms urine. When the kidneys fail, these by-products accumulate in the cells and the blood. If these products are not removed, the organism will die.

The kidney machine can be used to treat patients suffering from drug poisoning or kidney diseases such as uremia. This machine is an elaborate dialysis system that can be hooked up to the blood system. The blood is on one side of the dialysis membrane. A solution of glucose, ions, amino acids, and other compounds normally found in blood is present on the other side of the membrane. The blood contains large macromolecules such as enzymes and small biomolecules such as essential metabolites and the poisonous by-products of cellular metabolism. The large molecules cannot pass through the membrane. The small molecules can. The poisonous compounds that were originally on only one side of the membrane can pass through and are removed from the body.

The development of the kidney machine is a good example of how simple chemical and physical principles can be put to practical use by people. Of course, scientists make extensive use of this simple difference between large and small biomolecules when

they want to isolate and purify a component. One of the most active fields of biological research is concerned with the isolation of enzymes. Without procedures such as dialysis and other techniques that separate molecules on the basis of size, the functions of these biomolecules would still be a complete mystery.

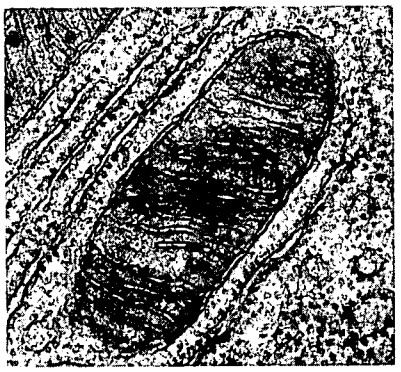
B-44 Mitochondria: Powerplants in Cells

One of the important subcellular organelles is the *mitochondrion*. Most cells contain many mitochondria. For example, liver cells contain hundreds. Bacterial cells are an exception. They have no mitochondria.

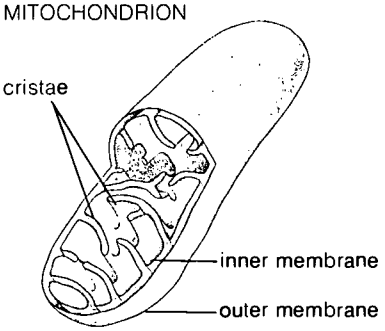
The wall of the mitochondrion is composed of two layers of membrane. The membranes contain important enzymes and cofactors. These enzymes and cofactors function in an extremely coordinated fashion to synthesize the high-energy compound ATP. The ATP produced is utilized by the cell in the multitude of biochemical reactions that require the input of energy. Because of this important role, the mitochondrion has been nicknamed the "powerhouse of the cell."

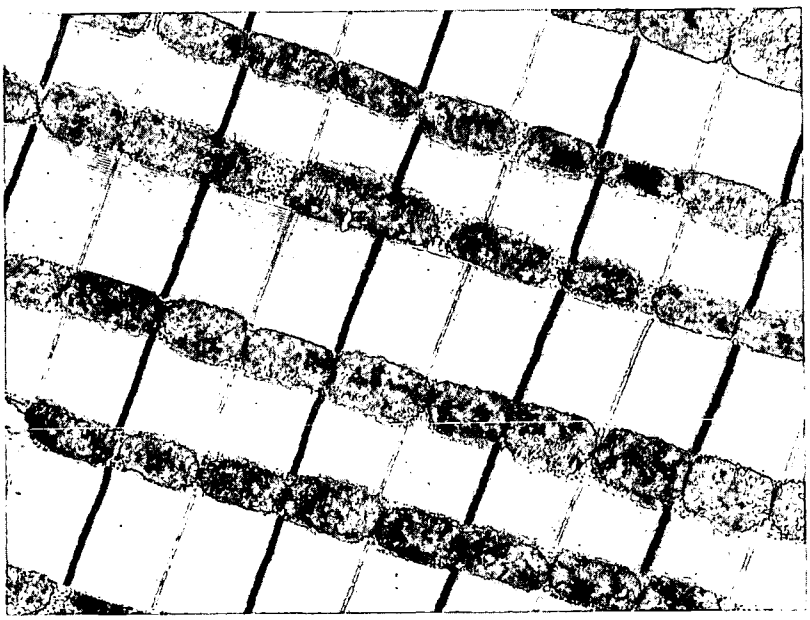
The mitochondrion itself actually has considerable structure. There is an outer membrane that encloses the mitochondrion and an inner membrane that is folded to form the *cristae* of the mitochondrion. You might wonder whether there are specific metabolic reactions associated with each of these membranes. There are. For example, succinate dehydrogenase and the respiratory chain enzymes are located in the inner membrane.

The mitochondrion is the "powerhouse" of the cell in which energy from organic molecules is made available for use in energy-requiring cell activities. A liquid-protein outer membrane surrounds the inner structure, which includes folded inner membranes called *cristae*.



MITOCHONDRION

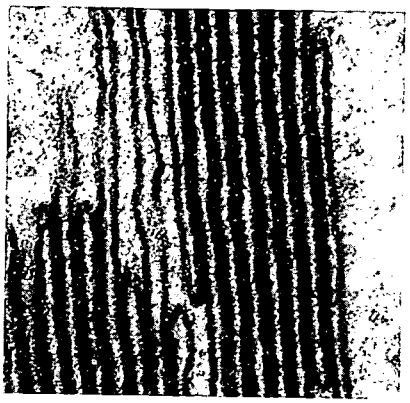




Mitochondria are the dark, oblong objects lined up end-to-end in the electron micrograph of a wasp's flight-muscle fiber. (Magnified approximately 9500 times.)

B-45 The Chloroplast and the Sun

An electron micrograph reveals the inner structure of chloroplasts. The chlorophyll molecules lie within a multi-layered sandwich of lipid-protein membranes, each of which is shown as a dark line.

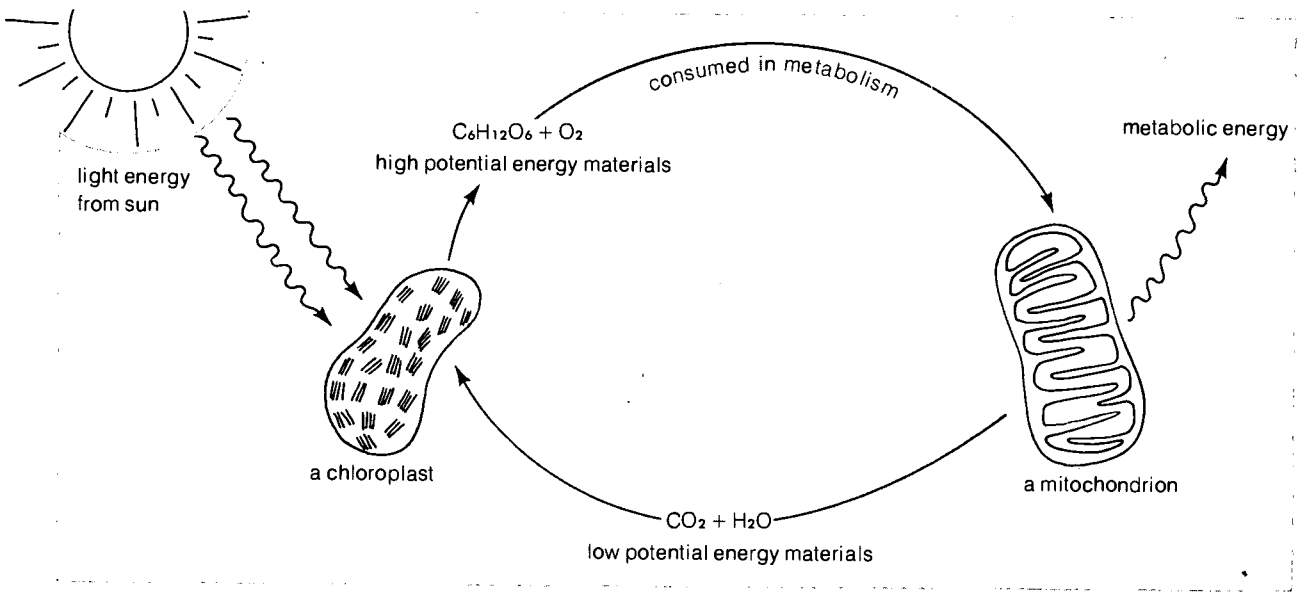


Plant cells contain organelles that are ultimately important to all living things. Because these organelles contain the green substance *chlorophyll,* they are called *chloroplasts.* These organelles convert the water and carbon dioxide from the environment into carbohydrates. Subsequently these compounds are converted into lipids, amino acids, and other molecules. Chloroplasts utilize light energy directly from the sun to synthesize these compounds. This process is called *photosynthesis.* (*Photo* literally means light.)

In some ways chloroplasts are similar to mitochondria. They both contain enzymatic pathways involved with energy. However, whereas the mitochondria are primarily involved in breaking down compounds to produce ATP, chloroplasts use light energy to build up compounds. All of the energy stored in molecules such as carbohydrates can be traced back to photosynthesis.

You might ask what would happen if there were no plants. If photosynthesis stopped, all living things would soon use up the available carbohydrates and life would end.

CONVERSION OF SOLAR ENERGY TO METABOLIC ENERGY



A chemical storehouse of energy is the glucose produced by green plants for their own use. However, the plants may be eaten by other living things for energy.

B-46 Separation of Subcellular Organelles

Thus far we have looked at three important cellular organelles. It is easy to see why they have been called "little organs." Each organelle has its own special duties, and somehow all the organelles cooperate to fulfill the function of the cell. But how do we know this? How do we know that the Krebs cycle enzymes are located

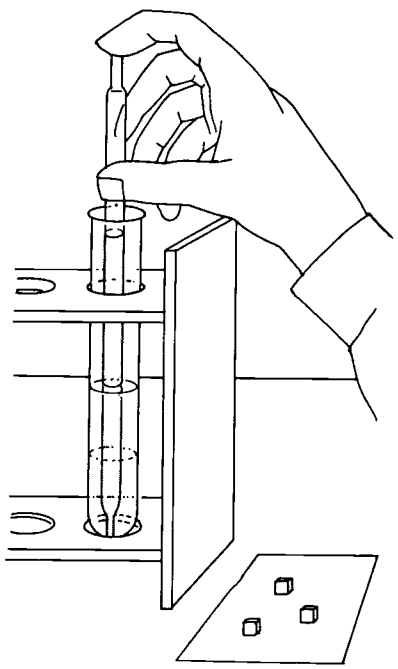
in the mitochondria? How do we know that photosynthesis takes place in the chloroplasts? How do biochemists determine which organelle contains which enzyme?

The method that is most useful is called *subcellular fractionation*. In this procedure, the cells are broken open with a device called a tissue grinder. As the cells are broken, the contents spill out. The subcellular organelles, such as the mitochondria, chloroplasts, and nuclei, are much stronger and more rugged than the whole cells. Thus, when special precautions are taken, these organelles do not break up after they are freed from the cells. In a way the process is similar to breaking an egg without breaking the yolk.

Once released from the cells, the various organelles can be separated from each other by *centrifugation*. This involves placing the suspension of broken cells in tubes within a centrifuge. In the centrifuge a force many times the force of gravity is generated by spinning the tubes at high speeds. As this force is applied to the broken cell suspension, the organelles and particles tend to be forced toward the bottom of the tube. This method takes advantage of the fact that subcellular organelles have different sizes and densities. The larger organelles such as the nuclei can be separated from the smaller ones such as the mitochondria because the larger ones sink farther and faster in the centrifuge tube.

miniexperiment

B-47 Subcellular Fractionation



The separation of subcellular organelles requires special equipment such as high-speed centrifuges. However, you do not need such equipment to understand the principles involved. In this experiment you will use plastic beads of different colors to represent different types of subcellular organelles. The principles that govern the separation of the beads in this experiment are the same principles that govern the separation of subcellular organelles.

Prepare a *sucrose gradient* by very carefully following these directions. Place 10 cm^3 of water in the bottom of your test tube. Fill your pipet with 10 cm^3 of 15-percent sucrose solution.

Keep your finger over the top hole in the pipet and carefully lower the pipet through the water until the pipet is in contact with the bottom of the test tube. By carefully adjusting your finger pressure over the top of the pipet, allow the sucrose solution to escape *very slowly* from the pipet. When the sucrose solution has drained out, carefully remove the pipet from the test tube. *Do not blow into the pipet while it is in the test tube.*

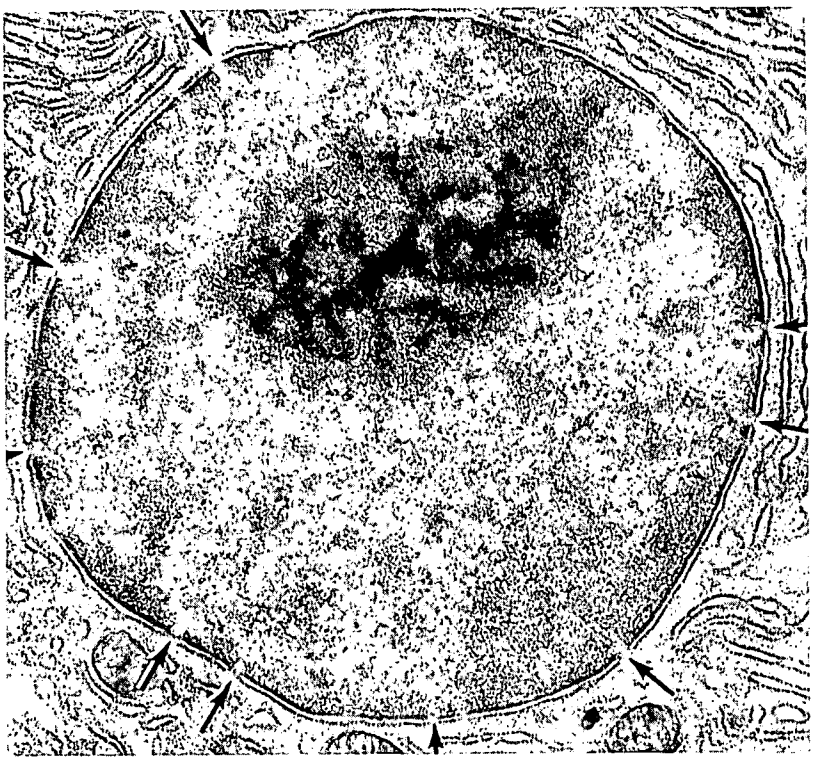
Fill the pipet with 10 cm^3 of 40-percent sucrose solution and add this solution to the test tube by repeating the procedure explained in the preceding paragraph.

The layers will remain separated from each other if the solutions are added very slowly and carefully. After you have prepared the gradient, carefully place each bead on top of the gradient using a spatula or a pair of forceps. You must be certain that the beads are dry, and air must not cling to them when they are placed in the gradient. Record your observations.

Questions:

1. What property of the beads is responsible for their behavior in the sucrose gradient?
2. Can you suggest a good name for this approach to the separation of subcellular organelles?

The technique of subcellular fractionation is a vital tool in the study of the chemistry of life. Using this technique in conjunction with the electron microscope, biochemists have begun to unravel the complex functions of subcellular organelles.



The nearly spherical cell nucleus is pitted with large pores (arrows). These pores allow material produced within the nucleus to move outward into the cell.

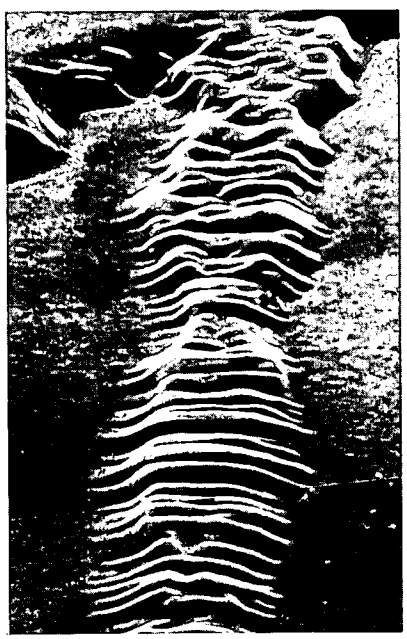
Genes (portions of DNA)
give rise to
↓
Enzymes and Proteins
catalyze reactions which result in
↓
Specific Traits
(such as eye color, height, etc.)

B-48 The Nucleus: Information Storehouse

The *nucleus* is the organelle that houses the genetic information of a cell. It contains the *chromosomes.* (The bacteria are exceptions. Their chromosomes are not contained within a nuclear membrane.) Each chromosome is a large body composed of *protein* and *deoxyribonucleic acid* (*DNA*). Scientists now know that the genetic information in a cell is actually contained in the DNA of the chromosomes.

Sections of the DNA along each chromosome correspond to units that are called *genes.* Each gene contains the hereditary information that the cell uses to synthesize the biomolecules necessary for a specific trait or characteristic. For example, you are probably familiar with the fact that eye color is an inherited trait. The color itself is dependent upon the synthesis of enzymes and proteins during the development of the iris of the eye. The synthesis of these enzymes and proteins can be traced back to the DNA in the genes themselves.

The DNA in the genes contains information in the nature of a code that is translated by the cell into proteins or enzymes. This is one use of DNA. It is also used to pass on genetic information to offspring. Thus, it is obvious that DNA is important. In order to understand the way in which DNA carries out its functions, we must understand its structure.



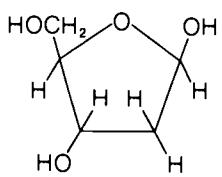
An electron micrograph of a replica of a small section of a chromosome from the salivary gland of the fruit fly drosophila.

B-49 Structure of Nucleic Acids

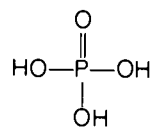
DNA is classified as a *nucleic acid*. Basically, there are two major types of nucleic acids: the *deoxyribonucleic acids* (*DNA*) and the *ribonucleic acids* (*RNA*). Both are large macromolecules composed of similar repeating units. Let us consider DNA first. In DNA each repeating unit is composed of three fundamental compounds: a monosaccharide called *deoxyribose*, a compound known as *phosphoric acid*, and *one* of four bases (adenine, cytosine, guanine, or thymine).

THE FUNDAMENTAL COMPONENTS OF DNA

DEOXYRIBOSE

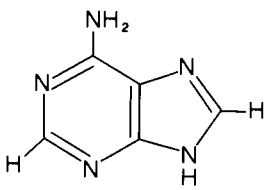


PHOSPHORIC ACID

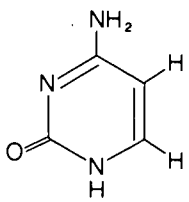


one of *four bases*

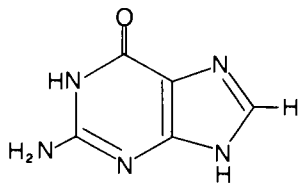
ADENINE



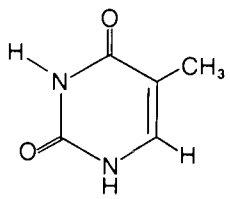
CYTOSINE



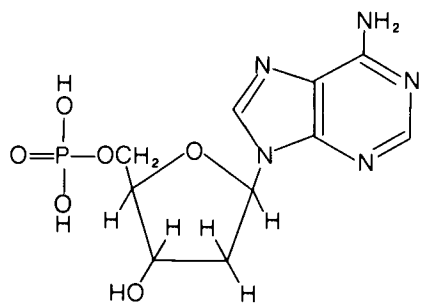
GUANINE



THYMINE

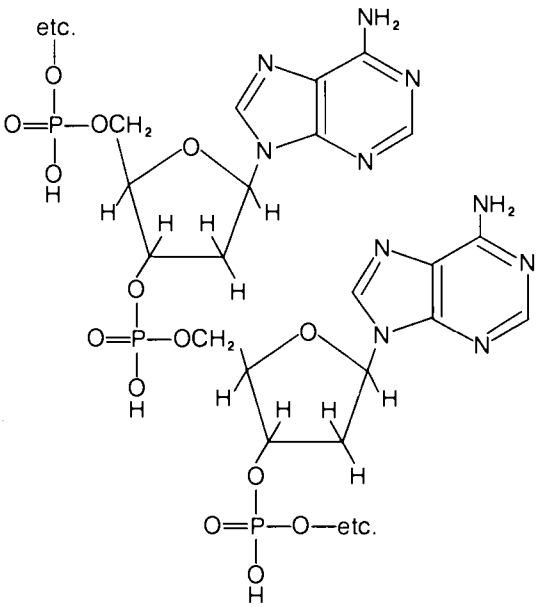


These compounds are put together to form a structure called a *nucleotide*.

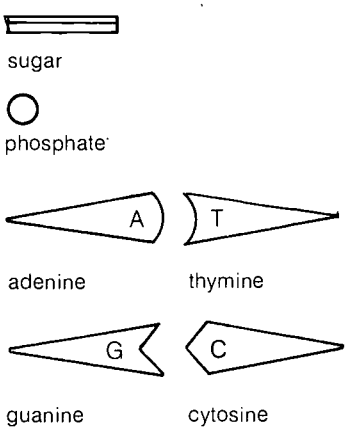


Because there are four bases, there are four nucleotides. The nucleotides are strung together to form the macromolecule known as DNA.

DNA

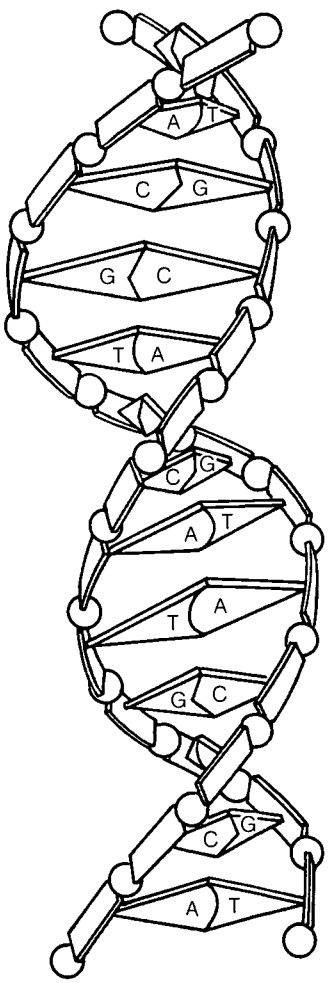


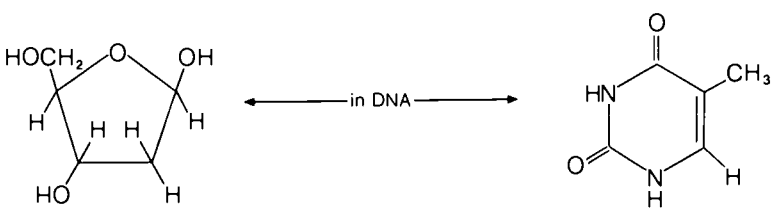
THE DNA MOLECULE

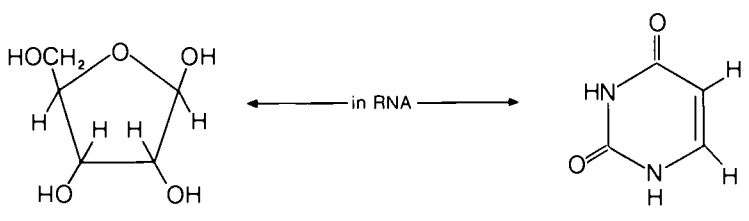


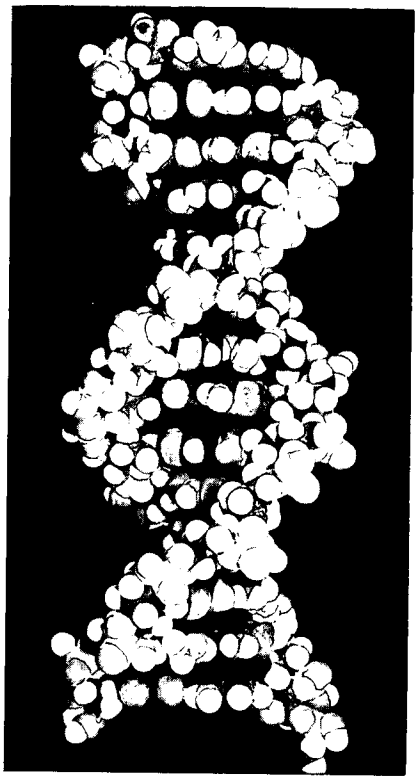
Ribonucleic acid (RNA) is composed of repeating units similar to those found in DNA. One difference is that *ribose* is the monosaccharide instead of deoxyribose. Also, RNAs have *uracil* in place of thymine. The RNA molecule is bonded together in the same way as DNA.

Although RNA molecules are large, they are quite small when compared with DNA. DNA molecules are extremely long, with molecular masses in the millions. Pieces of DNA have been found containing more than twenty thousand nucleotides.





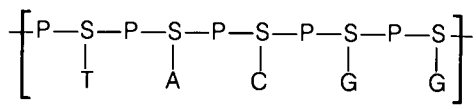




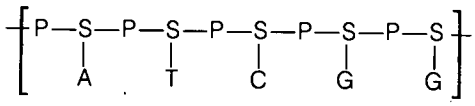
A model of DNA showing the double helix. In the double helix, two strands of DNA are coiled around each other. The strands are held together by nucleotide bases, giving the molecule a ladderlike structure.

The *sequence of nucleotides* in the DNA molecule is extremely important because the genetic information is contained in the nucleotide sequence in the form of a code. We will describe the code in section *B-51*, but first let us be sure we understand what a nucleotide sequence is.

When we discussed proteins in section *B-18*, we noted that different proteins contain different sequences of amino acids. Similarly, different DNA molecules contain different sequences of nucleotides. To see what this means, let us look more closely at two pieces of DNA.

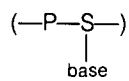


a nucleotide sequence



another nucleotide sequence

You can see that the pieces of DNA are the same length and have identical backbones composed of alternating phosphoric acid and sugar (—P—S—) molecules. However, you can also see that the two pieces of DNA are not the same; the bases attached to the sugar (—S—) molecules are arranged in a different order in the two pieces of DNA. That is, the sequence of nucleotides



in one piece of DNA is not the same as the sequence in the other. Can you make both nucleotide sequences the same by changing the position of only two bases? When the changes are made, both DNAs will have the same genetic information.

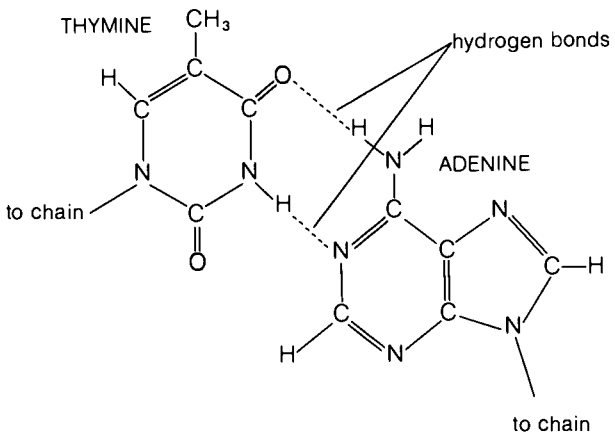
Now you know that each DNA molecule is a long string of nucleotides in a specific sequence. But what is the superstructure of DNA? What does the DNA look like in the chromosome? Actually DNA exists in the chromosome as *two long strands of DNA* molecules wound around each other in a spiral, or helix. Since two strands of DNA are involved and both strands are arranged in a helix, the structure is called the *double helix*.

James Watson and Francis Crick coined this term when they proposed that DNA existed as a coil, very much like a coiled spring. In the double helix the two strands are both coiled around each other, just as you can twist or coil two strands of string around each other. The two strands of DNA are intertwined in

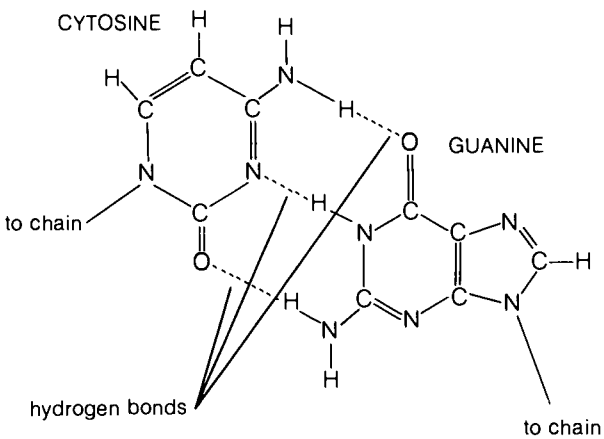
such a way that the resulting structure looks much like a spiral ladder. The sides of the spiral ladder consist of the phosphoric acid and deoxyribose (sugar) parts of the molecule. The rungs of the ladder are pairs of nucleotide bases.

What do we mean by pairs of nucleotide bases? Why don't the strands of DNA separate from each other? The answer to the first question provides an explanation for the second. Watson and Crick discovered that certain bases could be paired together very neatly because they form hydrogen bonds with each other. *In DNA, adenine (A) pairs only with thymine (T)* (A-T or T-A) and *guanine (G) pairs only with cytosine* (C) (G-C or C-G). In this way they form rungs of exactly the right length to hold the spiral ladder together.

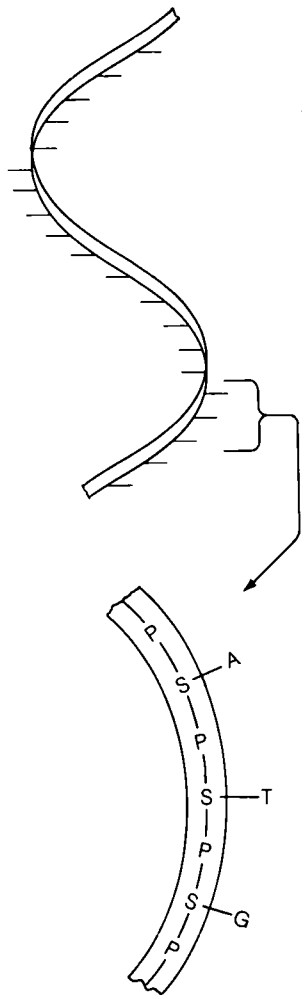
A Thymine-Adenine Base Pair



A Cytosine-Guanine Base Pair



A STRAND OF DNA



a nucleotide sequence

P = phosphate
S = sugar molecule (deoxyribose)

The Four Bases
G = guanine
C = cytosine
A = adenine
T = thymine

Francis Crick (*left*) and James D. Watson (*right*) worked as a team to discover that the DNA double helix was held together by hydrogen-bond base pairs.

Watson and Crick along with Maurice Wilkins received the Nobel Prize in 1962 for their work on the structure of DNA. Their concept of the double helix and the *hydrogen bonded base pairs* is considered a milestone in biochemistry. Using these concepts we can understand both the reproductive and informational role of DNA as we will explain in the next section.

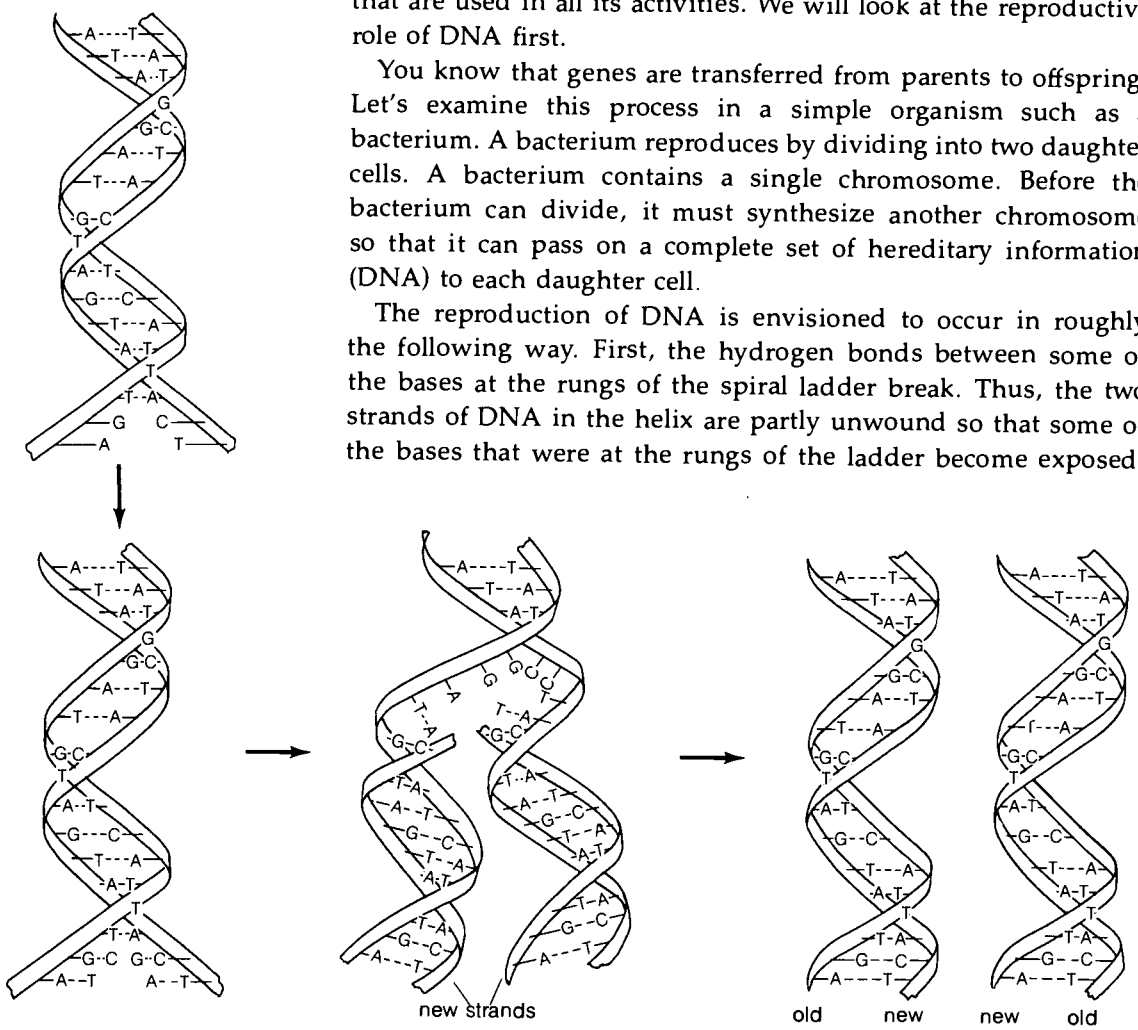
B-50 Double Role of the Double Helix

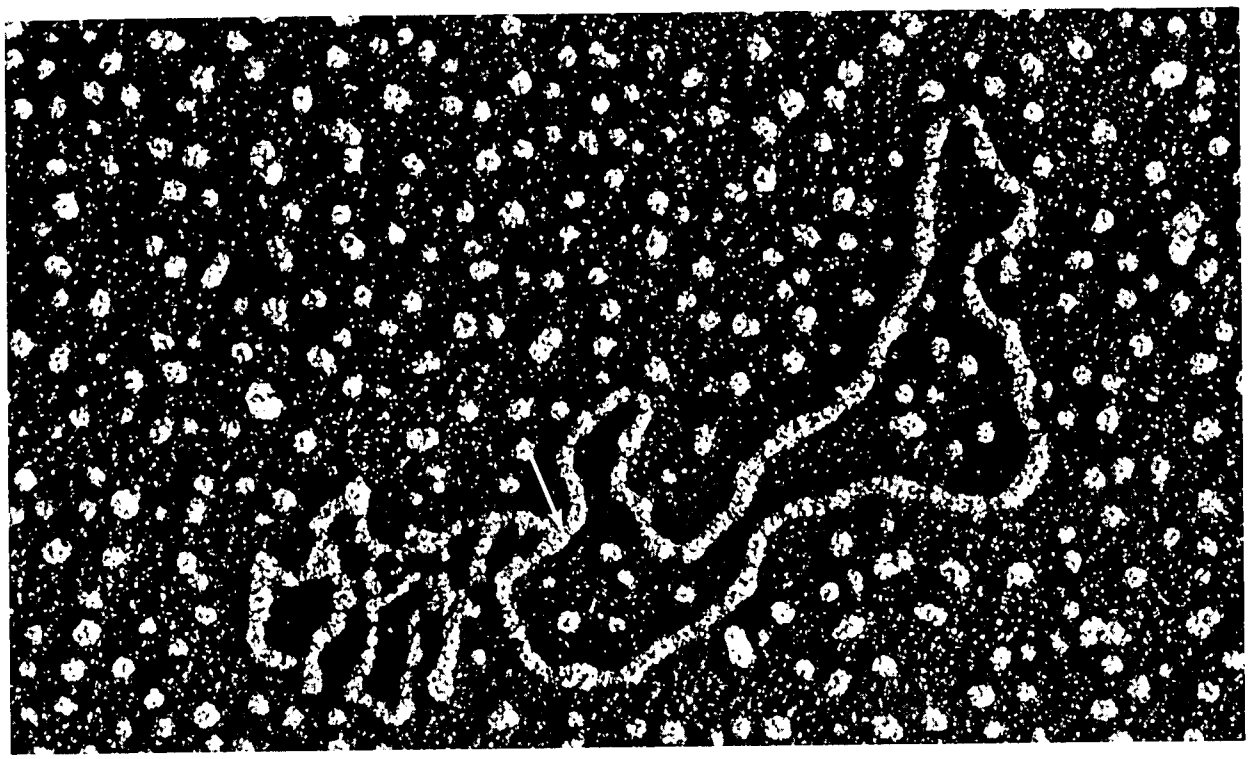
As we have said, DNA contains the genetic information of the cell. Because of this, DNA has a double role. First, it is used to reproduce a complete set of DNA molecules (genes) which are transferred from parents to offspring. This is the reproductive role of DNA. Second, the DNA is used by the cell for the synthesis of proteins and enzymes. In this role the coded information stored in DNA is translated by the cell to synthesize macromolecules that are used in all its activities. We will look at the reproductive role of DNA first.

You know that genes are transferred from parents to offspring. Let's examine this process in a simple organism such as a bacterium. A bacterium reproduces by dividing into two daughter cells. A bacterium contains a single chromosome. Before the bacterium can divide, it must synthesize another chromosome so that it can pass on a complete set of hereditary information (DNA) to each daughter cell.

The reproduction of DNA is envisioned to occur in roughly the following way. First, the hydrogen bonds between some of the bases at the rungs of the spiral ladder break. Thus, the two strands of DNA in the helix are partly unwound so that some of the bases that were at the rungs of the ladder become exposed.

THE REPRODUCTION OF DNA





This electron micrograph shows a replicating DNA molecule. Although the strands of the double helix cannot be seen, the junction at which the new DNA is being formed is clearly visible (arrow).

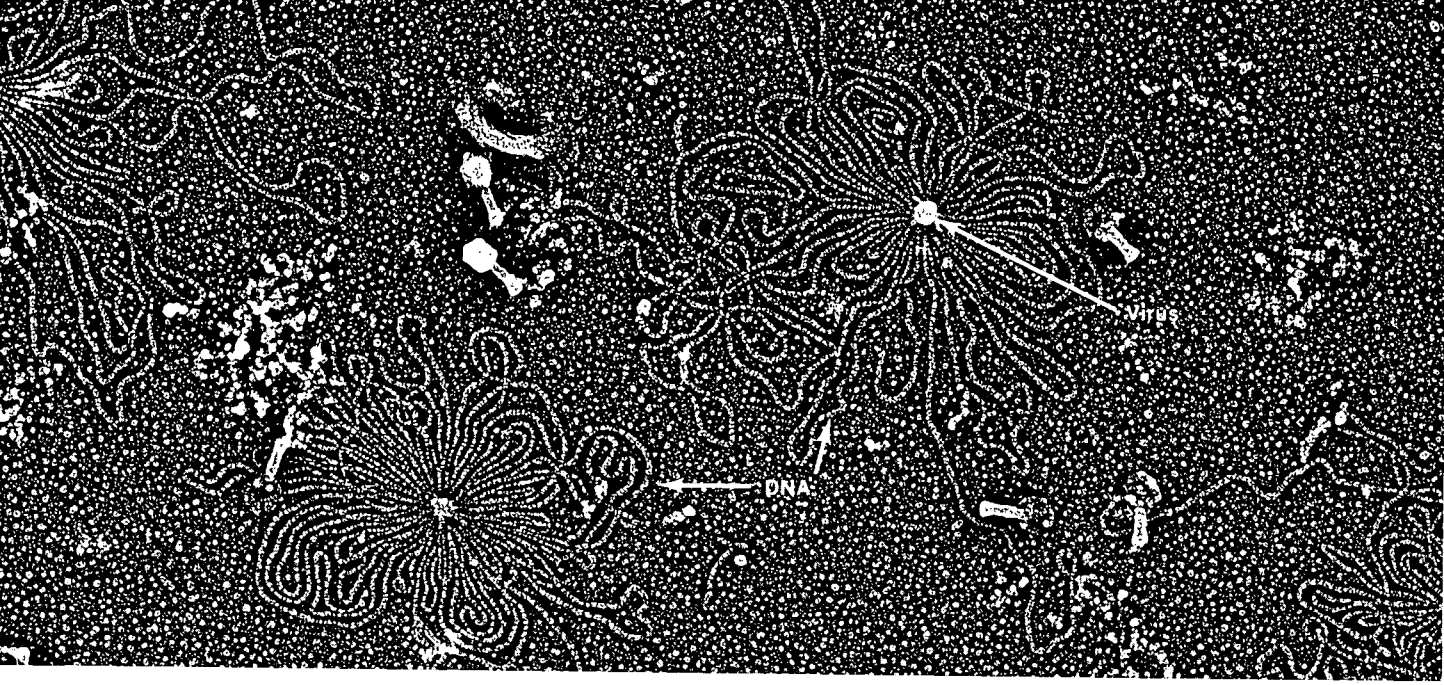
Then free nucleotides in the cell can pair with the appropriate bases that are exposed on both halves of the DNA molecule. Can you guess how they do this? As you might expect, they do this by forming hydrogen bonds. Finally, with the aid of a DNA synthesizing enzyme, the free nucleotides are connected to form the beginning of new strands of DNA. The enzyme continues to move along the original DNA strands until the cell has two identical DNA helices which are passed on to the daughter cells. Since these helices are identical, the daughter cells will have the same genetic information as the parent cell. This explains the reproductive role of DNA.

In the second role of DNA, the genetic information that is coded in the nucleotide sequence is used by the cell for the production of macromolecules. This process involves several steps.

Let us outline these steps before we consider them in detail. First, the information in the DNA is converted into a form that can leave the nucleus. This involves the synthesis of the second type of nucleic acid, RNA. In the nucleus the DNA actually helps to synthesize specific RNA molecules. As this is done, the information contained in the DNA sequence is transferred to the RNA molecules. The information in each RNA molecule is also contained in the nucleotide sequence. The molecules of RNA now contain the same coded messages that are in the DNA genes. Thus, these RNA molecules are called *messenger RNAs*.

TIME MACHINE

1952	Dwight D. Eisenhower is elected 34th president of the U.S.
1952	**Rosalind Franklin and R. G. Gosling obtain X-ray diffraction pictures, which lead to the discovery of the structure of DNA.**
1953	**James Watson and Francis Crick combine the ideas of base pairing and the double helix and obtain the correct structure of DNA.**
1954	Linus Pauling receives Nobel Prize in Chemistry for study of molecular forces.
1954	Bell Laboratories scientists develop first efficient solar battery.
1955	General Electric researchers produce synthetic diamonds.



An electron micrograph shows DNA that has been released from bacterial viruses. Normally, the DNA is tightly coiled within the virus. In order to reproduce, the virus must inject its DNA into a living cell.

Next the messenger RNAs leave the nucleus and travel to the ribosomes where the coded information is eventually translated into the amino acid sequences of proteins and enzymes. We will learn more about this in the next section. The proteins and enzymes are directly responsible for the hereditary traits that were originally coded in the form of DNA. Thus, the information in DNA is transferred to RNA and eventually used to synthesize protein.

How is the information in DNA transferred to RNA? When a gene is copied, RNA is synthesized by a process very similar to the way in which DNA was used to make new DNA. The two strands of the DNA helix partly unwind and expose some of the bases. Then free RNA nucleotides can hydrogen bond with the bases on the DNA and form base pairs. A special enzyme then links the RNA nucleotides to form a molecule of RNA. The new RNA separates from the DNA and leaves the nucleus. Finally, the strands of the DNA come together to reform the double helix. Thus, DNA is used as a template, or pattern, upon which a strand of RNA is formed.

DNA is actually used as a template for the synthesis of three different types of RNA molecules. These are called *ribosomal RNA*, *transfer RNA*, and *messenger RNA*. All of these RNAs leave the nucleus and come together at the ribosome where they are involved in the synthesis of proteins. However, they all have different functions.

The ribosomal and transfer RNA molecules do not carry any genetic information. The ribosomal RNA actually becomes part of the structure of the ribosome while the transfer RNA is used to transfer amino acids to the ribosome. Only the messenger RNA carries the coded genetic information to the ribosome.

Now you can see why DNA is often referred to as the master molecule. It has two very important functions. It acts as a template for the synthesis of new DNA so that genetic information can be passed down from generation to generation and it also acts as a template for the synthesis of RNA macromolecules which are used by the cell to synthesize proteins and enzymes.

B-51 Ribosomes and Protein Synthesis

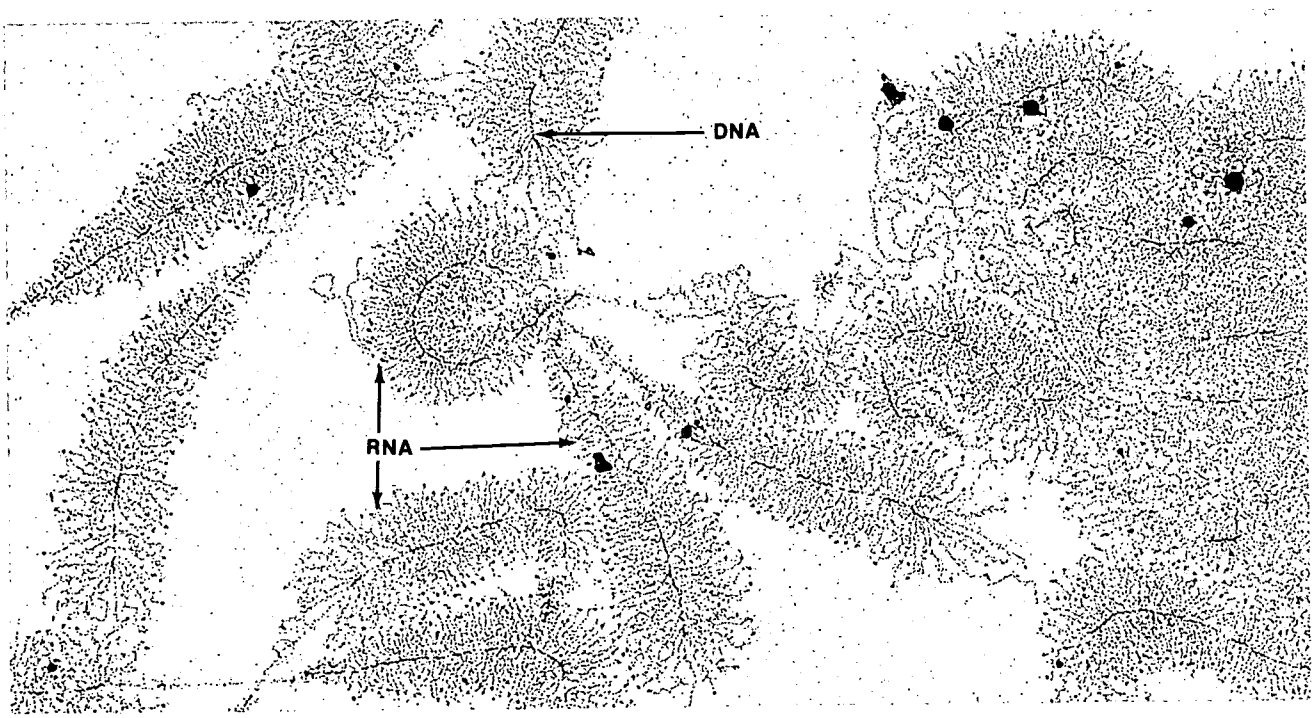
The ribosomes are subcellular particles composed of proteins and ribosomal RNA. The ribosome has been referred to as the "workbench of protein synthesis." At the ribosomal workbench the three types of RNA—ribosomal, transfer, and messenger—function in a coordinated way to synthesize proteins needed by the cell.

The ribosomal RNA actually becomes a part of the ribosomal workbench. The transfer RNAs carry amino acids to the ribosome and the messenger RNAs contain the coded genetic information in the form of nucleotide sequences. These sequences in messenger RNA are used to synthesize amino acid sequences in proteins. It is here at the ribosome that the coded message in messenger RNA is read and translated into specific protein molecules.

TIME MACHINE

1961	Major Yuri Gagarin (USSR) is the first man to orbit the earth.
1962	Lt. Col. John H. Glenn is the first American to orbit the earth.
1963	Lt. Valentina V. Tereshkova (USSR) is the first woman to orbit the earth.
1964	Dorothy M. Hodgkin receives Nobel Prize in Chemistry for determining structure of compounds (Vitamin B_{12}) needed in combating pernicious anemia.
1965	**Robert H. Holley and coworkers make the first determination of the sequence of an RNA molecule.**
1966	Miniskirts first appear in the United States.
1967	Christiaan Barnard of South Africa performs the first human heart transplant.
1968	Peggy Fleming wins Olympic women's figure skating title.
1969	Astronaut Neil Armstrong is the first man on the moon.

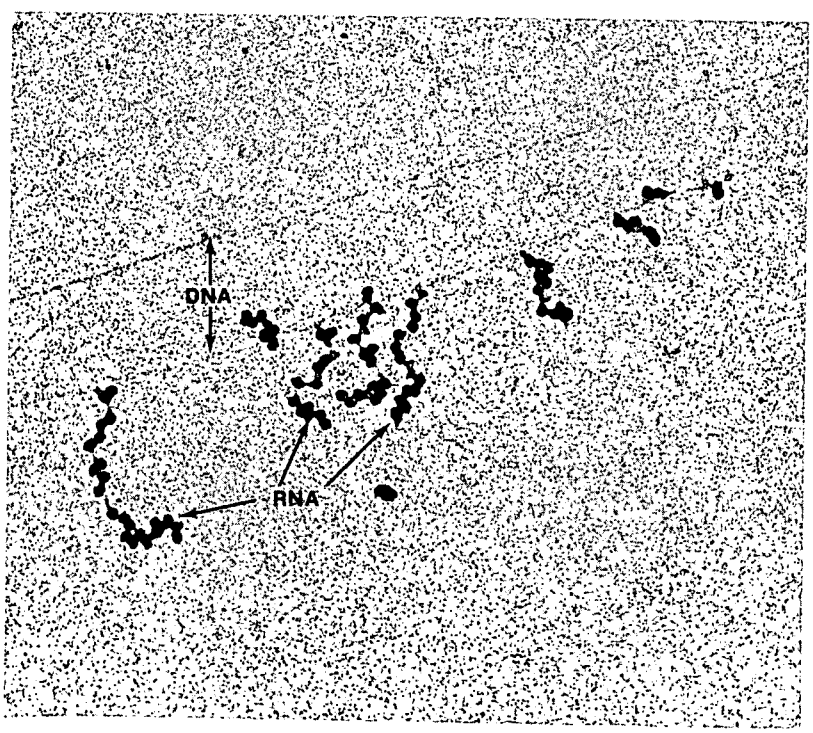
An electron micrograph shows the synthesis of hundreds of RNA strands along the longer (darker) strands of DNA.



We have said many times that the genetic information in these molecules is coded in the nucleotide sequence of nucleic acids. But what is the code? In a way the code is similar to our own language. Of course, we have twenty-six letters in our alphabet, whereas RNA contains only four—A, C, G, and U. Each word in the RNA language contains only three letters. In fact the RNA language is called the *triplet code*. Each three-letter word in the RNA language codes for a separate amino acid. For example, if the messenger RNA has the triplet sequence GGG, it codes for the amino acid glycine. Similarly, the three-letter word AAA codes for lysine.

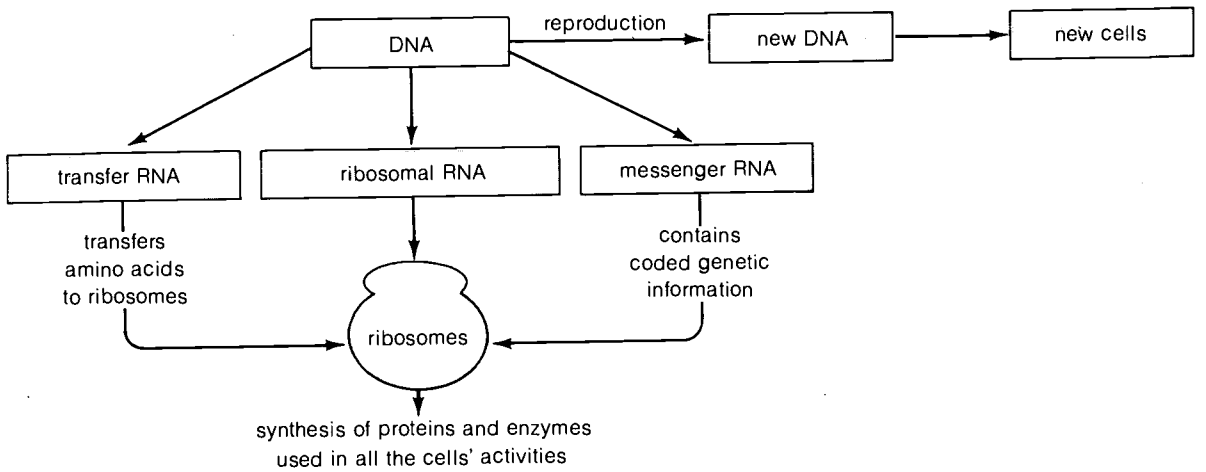
You might ask why each word has three letters and not two or four or more. You can figure this out for yourself. You know that there are twenty common amino acids. You also know that there are only four letters in the RNA language. If each letter codes for one amino acid, the four letters (bases) could code for only four amino acids. Thus, one-letter words will not work. If we used two-letter words, we could code more amino acids. How many? One word would be AA, another would be AU, and a third would be UA. You should be able to work out the rest.

Many biochemists have investigated the nature of the genetic code and have shown that it is a triplet code. It turns out that there are sixty-four different three-letter words in a four-letter alphabet. Since there are only twenty amino acids, there are more than enough three-letter words in the genetic language.



An electron micrograph reveals a bacterial gene in action. The upper strand is an inactive segment of a bacterial chromosome. The lower strand is active. DNA in the lower strand is being transcribed into messenger RNA by ribosomes (page 110), and the RNA is being translated into protein. Messenger RNA strands peel off toward the left. The longest strand (*left*) was the first to have been synthesized. Bacteria have no nucleus, and the ribosomes can interact with the messenger RNA as it is being synthesized.

THE DUAL ROLE OF DNA IN PROTEIN SYNTHESIS AND REPRODUCTION



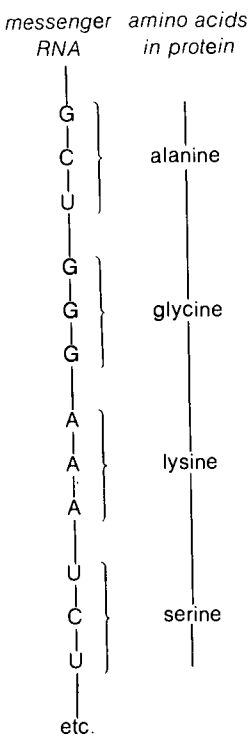
Now we can say that messenger RNA is a series of three-letter words in a specific sequence that corresponds to a specific sequence of amino acids in a protein. The role of messenger RNA is to carry the genetic information from the DNA in the nucleus to the ribosome. At the ribosomal workbench, the genetic information is translated into the exact sequence of amino acids in proteins.

At the ribosome the messenger RNA is fed along the ribosomal workbench much as you might thread a tape through the pick-up mechanism in a tape recorder. In this way only one triplet at a time is in the correct position to be decoded. As each triplet in the messenger RNA reaches this decoding area, it forms hydrogen bonds with a transfer RNA carrying the appropriate amino acid.

Each amino acid is carried to the ribosome by a unique transfer RNA molecule. Since there are twenty amino acids commonly found in proteins and enzymes, there are at least twenty different transfer RNAs. Each transfer RNA will hydrogen bond to a triplet on the messenger RNA, which codes for the appropriate amino acid. These hydrogen bonds are formed between the three-letter words on the messenger RNA and a matching triplet on the transfer RNA. This base pairing is the same as the base pairing that occurs in the synthesis of DNA and RNA.

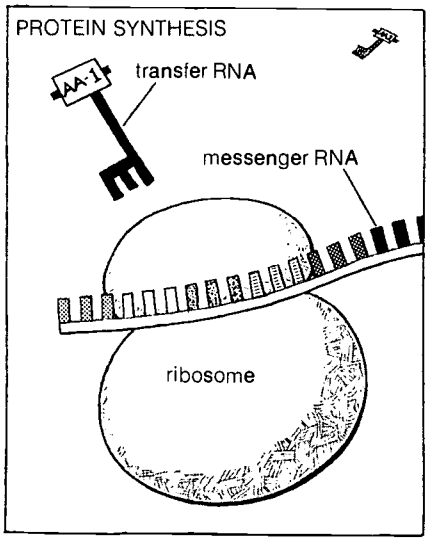
The synthesis of the protein at the ribosome is a stepwise process. First, a transfer RNA hydrogen bonds to a matching triplet on the messenger RNA. The amino acid carried by this transfer RNA will become the first amino acid of a new protein. Next the messenger RNA shifts along the ribosome so that the second triplet is in the correct position to hydrogen bond with another transfer RNA. This transfer RNA carries the second amino

RNA CODE SEQUENCE

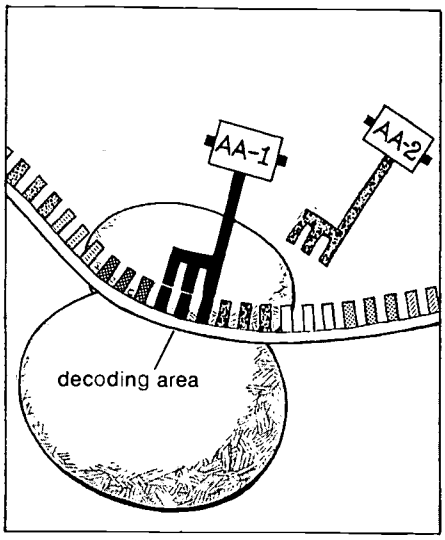


THE RNA ALPHABET

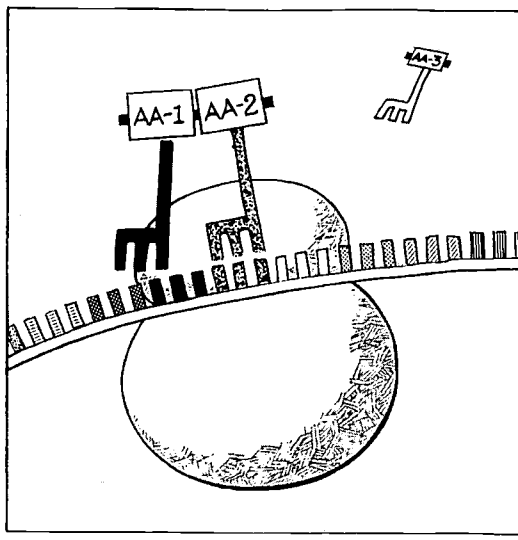
A = adenine
C = cytosine
G = guanine
U = uracil



Messenger RNA is led along the ribosomal workbench in the decoding area. The messenger RNA carries genetic information from the DNA in the nucleus.



A molecule of transfer RNA carries amino acid AA-1. The transfer RNA matches its AA-1 triplet to the messenger RNA triplet as hydrogen bonds form.



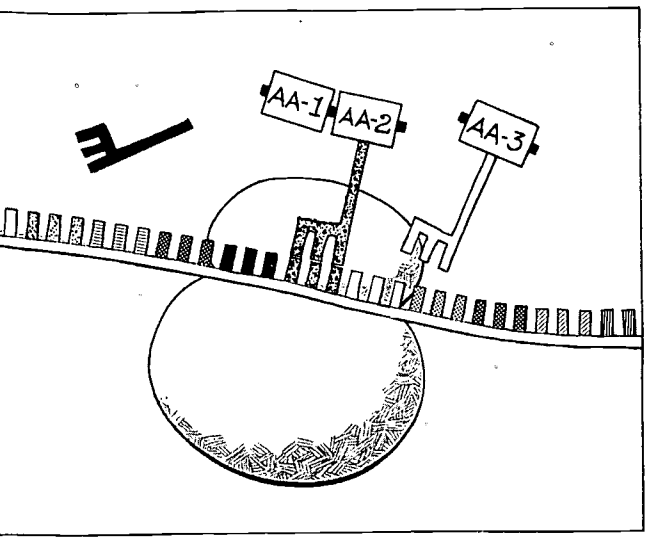
The messenger RNA moves along the workbench. A second transfer RNA hydrogen bonds to its matching triplet in the messenger RNA.

A photomicrograph magnifies the endoplasmic reticulum in a human cell. The micrograph reveals the ribosomes (arrows), which are embedded in the endoplasmic membrane.

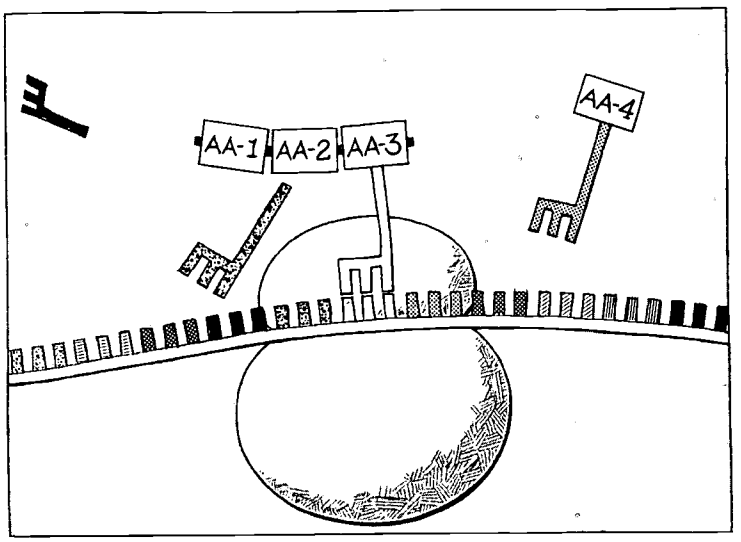
acid of the future protein. Then a protein-synthesizing enzyme connects the first amino acid to the second amino acid. As this is done, the first transfer RNA leaves the ribosome without its amino acid. The second transfer RNA remains hydrogen bonded to the messenger RNA and carries the growing protein.

Then the messenger RNA shifts again so that the next triplet is in place to hydrogen bond with a third transfer RNA. The growing protein chain is connected to the third amino acid. The second transfer RNA leaves, the messenger RNA shifts, and a fourth transfer RNA enters the decoding site. This process is repeated over and over again until the complete message is read. As each transfer RNA is matched to the messenger RNA, the amino acid on the transfer RNA is near the growing end of the protein chain. Thus, the amino acids are attached to the growing protein one by one in the same order as the code words appear in the messenger RNA.

The biosynthesis of proteins is a good example of the cooperation between different subcellular organelles. The synthesis of proteins requires RNA, amino acids, and energy. The RNA is synthesized in the nucleus under the direct control of the genes in DNA. The amino acids are attached to transfer RNA in the cytoplasm of the cell and carried to the ribosomes, where they are incorporated into proteins. The energy necessary for all of these metabolic activities is provided by ATP, which is synthesized in the mitochondria. The proteins that are synthesized are eventually used by the cell in its overall functions.



A protein-synthesizing enzyme connects amino acid AA-1 to amino acid AA-2. The bond is a peptide bond. The first transfer RNA leaves the ribosome.



The messenger RNA shifts in the decoding area. AA-3 and AA-2 are joined by a peptide bond. The protein continues to build as AA-4 approaches.

EXERCISES

1. Briefly describe the role of the following organelles:
   (a) the cell membrane
   (b) mitochondria
   (c) chloroplasts
   (d) nuclei
   (e) ribosomes
2. Which nucleotide base is unique to DNA?
3. Which nucleotide base is unique to RNA?
4. Compare the structures of thymine and uracil. When RNA is synthesized by base pairing with DNA, do you think that uracil pairs with adenine, thymine, guanine, or cytosine? Explain.
5. If one strand of DNA had the sequence A-T-C-A-T-G, what would the sequence be in the other strand of the DNA helix?
6. If a portion of DNA in a gene had the sequence A-T-C-A-G, what would the nucleotide sequence be in the messenger RNA synthesized from this DNA sequence?
7. The enzyme ribonuclease has 124 amino acids. How many nucleotides would be required in the gene for ribonuclease to code for these 124 amino acids?
8. If DNA contained only two kinds of bases, could a triplet code be used for the 20 common amino acids? Explain.

Where Are We?

In this module we have seen that biochemists usually work at the molecular level. You have learned that many properties of living things can be understood on the basis of relatively simple chemical and physical principles. These principles not only explain the properties of simple molecules, but they also help to explain the complex processes in our own bodies.

Instruments such as the electron microscope help scientists in finding out more about biomolecules. Greater magnifications can now be attained by using these instruments in scientific research.

For instance, some diseases are the result of very simple biochemical changes. A good example is the disease sickle-cell anemia, which is a genetic disease. The name comes from the fact that the red blood cells of people with the disease have a sickle shape rather than the normal disc shape. This happens because the hemoglobin in the sickle cells precipitates into long rods which push the red blood cells into the sickle shape from the inside.

The sequence of amino acids in sickle-cell hemoglobin is different from the sequence in normal hemoglobin. When the sickle-cell hemoglobin is synthesized, the amino acid valine is incorporated instead of glutamate in one position in the chain. In normal hemoglobin, the sixth amino acid from the end of the chain is glutamate, while in sickle-cell hemoglobin the amino acid valine is found in this position. This small difference in amino acid sequence results in a drastic difference in the solubilities of these two types of hemoglobin.

The change in the amino acid sequence results from a change in the DNA nucleotide sequence. The DNA triplet that codes for glutamate (CTT) in the normal gene is changed to the triplet that codes for valine (CAT) in the gene for sickle-cell hemoglobin. Thus, a change in a single nucleotide in DNA is responsible for a crippling disease. Such a change in DNA is called a *mutation*. At this time it is virtually impossible to cure a genetic disease because there is no way to introduce new genes into humans to replace defective genes.

However, methods for transferring genes between organisms are being developed, and it is now possible to transfer mammalian genes (DNA) into bacteria. This DNA is called recombinant DNA

A normal red blood cell shows up in a photomicrograph (*left*) as a circular corpuscle. Red blood cells become sickled (*right*) when hemoglobin precipitates into long rods, causing sickle-cell anemia. Single sickle-cell *(far right)* magnified 1250 times.

Recombinant DNA techniques allow biologists to determine where a gene is located in a chromosome and how many copies there are. The groups of black dots indicate where radioactive copies of a single gene matched (paired) the DNA in these chromosomes.

This stamp commemorates the isolation of insulin by Canadian researchers in 1921. This discovery led to a treatment for diabetes that has not yet been effectively replaced by synthetic drugs or any other measures. It also led to a Nobel prize for two of the researchers involved.

because the specific piece of DNA making up the gene is removed from one organism and recombined with the DNA of another. Many scientists feel that it will soon be possible to have bacteria produce many human enzymes and hormones that can be used for medical treatment.

For example, many diabetics need daily injections of the hormone insulin. This hormone is presently extracted from pig or beef pancreas. Insulin is expensive and some people become allergic to it. Scientists plan to insert the human insulin gene into bacteria so that the bacteria can produce the hormone. Thus, it would be possible to produce the hormone cheaply using fermentation methods.

However, the idea of transferring genes from one organism to another is very controversial. Even though DNA recombination may lead to many benefits for medicine and agriculture, some people fear that new and dangerous organisms will be produced. Because of this possibility, scientists who do this work are required to take many precautions to prevent any danger to the public.

Oddly enough, biochemists have recently found that bacteria in the "wild" transfer genes from one species to another. For example, a gene found in some bacteria makes them resistant to certain antibiotics. The gene seems to have been transferred from the intestinal bacterium, *E. coli*, to the gonorrhea bacterium, the *gonococcus*. Thus, bacteria may have been involved in their own recombinant DNA experiments all along. However, caution is needed in experiments conducted by scientists because the true benefits and dangers of recombinant DNA have yet to be found.

Of course, biochemistry provides insight and solutions for many problems outside of medicine. For example, biochemistry has played an important role in the improvement of agriculture. You can understand why phosphate is an important part of fertilizers. It is required for metabolism and forms a part of nucleic acids. Similarly, ammonia and other nitrogen-containing compounds are also important in plant biochemistry because they are needed for the synthesis of proteins and nucleic acids. Unfortunately, plants cannot utilize the large amount of N_2 present in our atmosphere. A few kinds of plants have bacteria present in their roots that can convert atmospheric N_2 to ammonia. This process is called *nitrogen fixation*. If we could improve our understanding of the biochemistry of this process, we might be able to introduce the bacteria into other plants. This would help to reduce our need for nitrogen-containing fertilizers.

While many problems in the area of biochemistry have been solved, many questions have yet to be answered. We've come a long way in a short time, but we have a long way to go. And that's where we are!

Appendix I: Safety

SAFETY IN THE LABORATORY

Proper conduct in a chemistry laboratory is really an extension of safety procedures normally followed each day around your home and in the outside world. Exercising care in a laboratory demands the same caution you apply to driving a car, riding a motorbike or bicycle, or participating in a sport. Athletes consider safety measures a part of playing the game. For example, football players willingly spend a great deal of time putting on equipment such as helmets, hip pads, and shoulder pads to protect themselves from potential injury.

Chemists must also be properly dressed. To protect themselves in the laboratory, they commonly wear a lab apron or a coat and protective glasses. Throughout this course you will use similar items. Hopefully their use will become second nature to you, much as it becomes second nature for a baseball catcher to put on a chest protector and mask before stepping behind home plate.

As you read through a written experimental procedure, you will notice that specific hazards and precautions are called to your attention. Be prepared to discuss these hazards with your teacher and with your fellow students. Always read the entire experimental procedure thoroughly before starting any laboratory work.

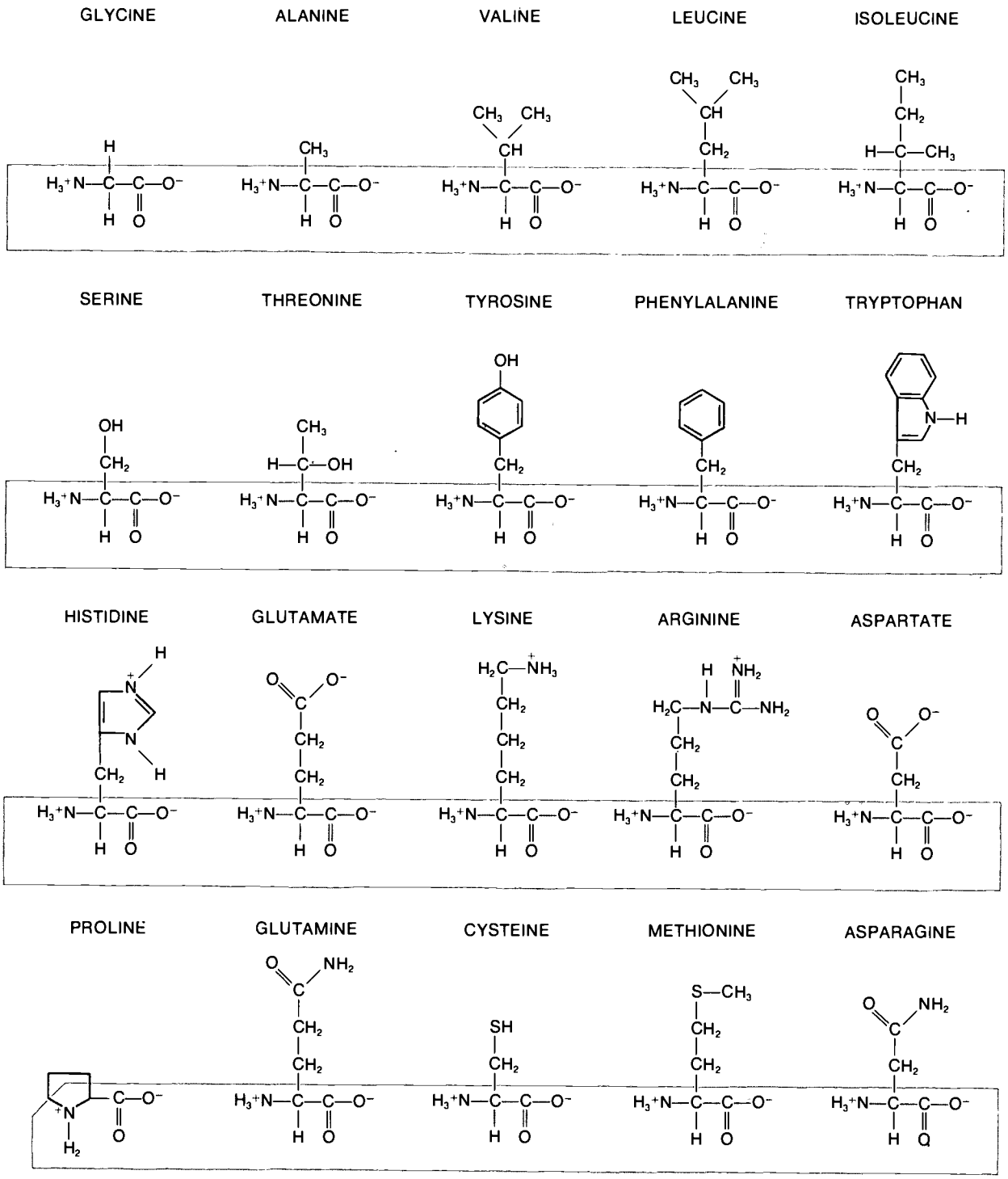
A list of general laboratory safety procedures follows. It is not intended that you memorize these safety procedures but rather that you *use* them regularly when performing experiments. You may notice that this list is by no means complete. Your teacher may wish to add safety guidelines that are relevant to your specific classroom situation. It would be impossible to anticipate every hazardous situation that might arise in the chemistry laboratory. However, if you are familiar with these general laboratory safety procedures and if you use common sense, you will be able to handle potentially hazardous situations intelligently and safely. Treat all chemicals with respect, not fear.

GENERAL SAFETY GUIDELINES

1. Work in the laboratory only when the teacher is present or when you have been given permission to do so. In case of accident, notify your teacher immediately.
2. Before starting any laboratory exercise, be sure that the laboratory bench is clean.
3. Put on a laboratory coat or apron and protective glasses or goggles before beginning an experiment.
4. Tie back loose hair to prevent the possibility of its contacting any Bunsen burner flames.
5. Open sandals or bare feet are not permitted in the laboratory. The dangers of broken glass and corrosive liquid spills are always present in a laboratory.
6. Fire is a special hazard in the laboratory because many chemicals are flammable. Learn how to use the fire blanket, fire extinguisher, and shower (if your laboratory has one).
7. For minor skin burns, immediately immerse the burned area in cold water for several minutes. Then consult your teacher for further instructions on possible additional treatment.
8. In case of a chemical splash on your skin, immediately rinse the area with cold water for at least one minute. Consult your teacher for further action.
9. If any liquid material splashes into your eye, wash the eye immediately with water from an eyewash bottle or eyewash fountain.
10. Never look directly down into a test tube—view the contents of the tube from the side. (Why?)
11. Never smell a material by placing your nose directly at the mouth of the tube or flask. Instead, with your hand, "fan" some of the vapor from the container toward your nose. Inhale cautiously.
12. Never taste any material in the laboratory.
13. Never add water to concentrated acid solutions. The heat generated may cause spattering. Instead, as you stir, add the acid slowly to the water or dilute solution.
14. Read the label on a chemical bottle at least *twice* before removing a sample. H_2O_2 is not the same as H_2O.
15. Follow your teacher's instructions or laboratory procedure when disposing of used chemicals.

This symbol represents three of the common hazards in a chemistry laboratory—flame, fumes, and explosion. It will appear with certain experiments in this module to alert you to special precautions in addition to those discussed in this Appendix.

Appendix II: 20 Common Amino Acids



The 20 amino acids illustrated here are commonly found in proteins. Each has a different R group or side chain, attached to the central carbon atom, so that the various amino acids exhibit a wide range of chemical properties. The amino acids are shown in the ionic forms present in biological systems.

Appendix III: Metric Units

PHYSICAL QUANTITY	SI BASE OR DERIVED UNIT		OTHER UNITS	
	NAME	SYMBOL AND DEFINITION	NAME	SYMBOL AND DEFINITION
length	meter*	m	kilometer centimeter nanometer	1 km = 10^3 m 1 cm = 10^{-2} m 1 nm = 10^{-9} m = 10^{-7} cm
area	square meter	m^2	square centimeter	1 cm^2 = 10^{-4} m^2
volume	cubic meter	m^3	cubic centimeter liter	1 cm^3 = 10^{-6} m^3 1 l = 10^3 cm^3
mass	kilogram*	kg	gram	1 g = 10^{-3} kg
time	second*	s		
amount of substance	mole*	mol	millimole micromole nanomole	1 m mol = 10^{-3} mol 1 μ mol = 10^{-6} mol 1 n mol = 10^{-9} mol
concentration	moles per cubic meter	mol/m^3	moles per liter molar concentration (molarity)	1 mol/l = 10^3 mol/m^3 1 M = mol/l
Celsius temperature			degree Celsius	°C

*SI base unit, exactly defined in terms of certain physical measurements.

Selected Readings

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A highly readable account of this complex chemical process on which all life as we know it depends.

———. *The Chemicals of Life.* New York: The New American Library, Inc., 1962. Paperback.
An account of how enzymes, vitamins, and hormones control the functions of living cells; how deficiency diseases arise; and how wonder drugs work.

———. *The Wellsprings of Life.* New York: The New American Library, Inc., 1960. Paperback.
The author examines the structure of the cell and the biochemistry of its food and fuel processes as he considers the question "Where does life come from?"

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In addition, discussion of new developments in chemistry relevant to the topics covered in this module will be found in *Chemistry, Journal of Chemical Education, Scientific American*, and *Science News*.

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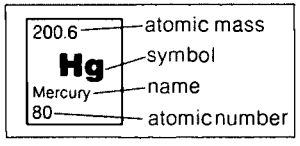
TABLE OF INTERNATIONAL RELATIVE ATOMIC MASSES*

Element	Symbol	Atomic Number	Atomic Mass
Actinium	Ac	89	227.0
Aluminum	Al	13	27.0
Americium	Am	95	(243)**
Antimony	Sb	51	121.8
Argon	Ar	18	39.9
Arsenic	As	33	74.9
Astatine	At	85	(210)
Barium	Ba	56	137.3
Berkelium	Bk	97	(247)
Beryllium	Be	4	9.01
Bismuth	Bi	83	209.0
Boron	B	5	10.8
Bromine	Br	35	79.9
Cadmium	Cd	48	112.4
Calcium	Ca	20	40.1
Californium	Cf	98	(251)
Carbon	C	6	12.0
Cerium	Ce	58	140.1
Cesium	Cs	55	132.9
Chlorine	Cl	17	35.5
Chromium	Cr	24	52.0
Cobalt	Co	27	58.9
Copper	Cu	29	63.5
Curium	Cm	96	(247)
Dysprosium	Dy	66	162.5
Einsteinium	Es	99	(254)
Erbium	Er	68	167.3
Europium	Eu	63	152.0
Fermium	Fm	100	(257)
Fluorine	F	9	19.0
Francium	Fr	87	(223)
Gadolinium	Gd	64	157.3
Gallium	Ga	31	69.7
Germanium	Ge	32	72.6
Gold	Au	79	197.0
Hafnium	Hf	72	178.5
Helium	He	2	4.00
Holmium	Ho	67	164.9
Hydrogen	H	1	1.008
Indium	In	49	114.8
Iodine	I	53	126.9
Iridium	Ir	77	192.2
Iron	Fe	26	55.8
Krypton	Kr	36	83.8
Lanthanum	La	57	138.9
Lawrencium	Lr	103	(260)
Lead	Pb	82	207.2
Lithium	Li	3	6.94
Lutetium	Lu	71	175.0
Magnesium	Mg	12	24.3
Manganese	Mn	25	54.9
Mendelevium	Md	101	(258)
Mercury	Hg	80	200.6
Molybdenum	Mo	42	95.9
Neodymium	Nd	60	144.2
Neon	Ne	10	20.2
Neptunium	Np	93	237.0
Nickel	Ni	28	58.7
Niobium	Nb	41	92.9
Nitrogen	N	7	14.0
Nobelium	No	102	(259)
Osmium	Os	76	190.2
Oxygen	O	8	16.0
Palladium	Pd	46	106.4
Phosphorus	P	15	31.0
Platinum	Pt	78	195.1
Plutonium	Pu	94	(244)
Polonium	Po	84	(209)
Potassium	K	19	39.1
Praseodymium	Pr	59	140.9
Promethium	Pm	61	(145)
Protactinium	Pa	91	231.0
Radium	Ra	88	226.0
Radon	Rn	86	(222)
Rhenium	Re	75	186.2
Rhodium	Rh	45	102.9
Rubidium	Rb	37	85.5
Ruthenium	Ru	44	101.1
Samarium	Sm	62	150.4
Scandium	Sc	21	45.0
Selenium	Se	34	79.0
Silicon	Si	14	28.1
Silver	Ag	47	107.9
Sodium	Na	11	23.0
Strontium	Sr	38	87.6
Sulfur	S	16	32.1
Tantalum	Ta	73	180.9
Technetium	Tc	43	(97)
Tellurium	Te	52	127.6
Terbium	Tb	65	158.9
Thallium	Tl	81	204.4
Thorium	Th	90	232.0
Thulium	Tm	69	168.9
Tin	Sn	50	118.7
Titanium	Ti	22	47.9
Tungsten	W	74	183.8
Uranium	U	92	238.0
Vanadium	V	23	50.9
Xenon	Xe	54	131.3
Ytterbium	Yb	70	173.0
Yttrium	Y	39	88.9
Zinc	Zn	30	65.4
Zirconium	Zr	40	91.2

*Based on International Union of Pure and Applied Chemistry (IUPAC) values (1975).

**Numbers in parentheses give the mass numbers of the most stable isotopes.

PERIODIC TABLE OF THE ELEMENTS



IA	IIA							VIIIB			IB	IIB	IIIA	IVA	VA	VIA	VIIA	VIIIA
																		4.00 **He** Helium 2
6.94 **Li** Lithium 3	9.01 **Be** Beryllium 4												10.8 **B** Boron 5	12.0 **C** Carbon 6	14.0 **N** Nitrogen 7	16.0 **O** Oxygen 8	19.0 **F** Fluorine 9	20.2 **Ne** Neon 10
23.0 **Na** Sodium 11	24.3 **Mg** Magnesium 12	IIIB	IVB	VB	VIB	VIIB							27.0 **Al** Aluminum 13	28.1 **Si** Silicon 14	31.0 **P** Phosphorus 15	32.1 **S** Sulfur 16	35.5 **Cl** Chlorine 17	39.9 **Ar** Argon 18
39.1 **K** Potassium 19	40.1 **Ca** Calcium 20	45.0 **Sc** Scandium 21	47.9 **Ti** Titanium 22	50.9 **V** Vanadium 23	52.0 **Cr** Chromium 24	54.9 **Mn** Manganese 25	55.8 **Fe** Iron 26	58.9 **Co** Cobalt 27	58.7 **Ni** Nickel 28	63.5 **Cu** Copper 29	65.4 **Zn** Zinc 30		69.7 **Ga** Gallium 31	72.6 **Ge** Germanium 32	74.9 **As** Arsenic 33	79.0 **Se** Selenium 34	79.9 **Br** Bromine 35	83.8 **Kr** Krypton 36
85.5 **Rb** Rubidium 37	87.6 **Sr** Strontium 38	88.9 **Y** Yttrium 39	91.2 **Zr** Zirconium 40	92.9 **Nb** Niobium 41	95.9 **Mo** Molybd'm 42	(97) **Tc** Technetium 43	101.1 **Ru** Ruthenium 44	102.9 **Rh** Rhodium 45	106.4 **Pd** Palladium 46	107.9 **Ag** Silver 47	112.4 **Cd** Cadmium 48		114.8 **In** Indium 49	118.7 **Sn** Tin 50	121.8 **Sb** Antimony 51	127.6 **Te** Tellurium 52	126.9 **I** Iodine 53	131.3 **Xe** Xenon 54
132.9 **Cs** Cesium 55	137.3 **Ba** Barium 56	138.9 **La*** Lanthanum 57	178.5 **Hf** Hafnium 72	180.9 **Ta** Tantalum 73	183.8 **W** Tungsten 74	186.2 **Re** Rhenium 75	190.2 **Os** Osmium 76	192.2 **Ir** Iridium 77	195.1 **Pt** Platinum 78	197.0 **Au** Gold 79	200.6 **Hg** Mercury 80		204.4 **Tl** Thallium 81	207.2 **Pb** Lead 82	209.0 **Bi** Bismuth 83	(209) **Po** Polonium 84	(210) **At** Astatine 85	(222) **Rn** Radon 86
(223) **Fr†** Francium 87	226.0 **Ra** Radius 88	(227) **Ac**** Actinium 89	‡ 104	‡ 105	‡ 106													

*	140.1 **Ce** Cerium 58	140.9 **Pr** Praseody'm 59	144.2 **Nd** Neodymium 60	(145) **Pm** Promethium 61	150.4 **Sm** Samarium 62	152.0 **Eu** Europium 63	157.3 **Gd** Gadolinium 64	158.9 **Tb** Terbium 65	162.5 **Dy** Dysprosium 66	164.9 **Ho** Holmium 67	167.3 **Er** Erbium 68	168.9 **Tm** Thulium 69	173.0 **Yb** Ytterbium 70	175.0 **Lu** Lutetium 71
**	232.0 **Th** Thorium 90	231.0 **Pa** Protactinium 91	238.0 **U** Uranium 92	237.0 **Np** Neptunium 93	(242) **Pu** Plutonium 94	(243) **Am** Americium 95	(245) **Cm** Curium 96	(245) **Bk** Berkelium 97	(251) **Cf** Californium 98	(254) **Es** Einsteinium 99	(254) **Fm** Fermium 100	(256) **Md** Mendel'm 101	(254) **No** Nobelium 102	(257) **Lr** Lawrencium 103

†The most stable known isotopes are shown in parentheses.

‡The discovery of elements 104, 105, and 106 has been claimed by both American and Soviet scientists. The Americans have suggested the name *rutherfordium* and *hahnium* for 104 and 105; the Soviets have suggested the names *kurchatovium* and *nielsbohrium* for these same elements. No name has yet been proposed for element 106.

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